RAFFINOSE SYNTHASE GENES AND THEIR USE

BACKGROUND OF THE INVENTION

FIELD OF INVENTION

The present invention relates to raffinose synthase genes and their use.

DISCLOSURE OF THE RELATED ART

Raffinose family oligosaccharides are derivatives of sucrose, which are represented by the general formula: o-α-D-galactopyranosyl-(1→6)$_n$-o-α-D-glucopyranosyl-(1→2)-β-D-fluctofuranoside, and they are called "raffinose" when n is 1, "stachyose" when n is 2, "verbascose" when n is 3, and "ajugose" when n is 4.

It has been known that raffinose family oligosaccharides have an effect of giving good conditions of enterobacterial flora, if present at an appropriate amount in food. Therefore, raffinose family oligosaccharides have already been used as a functional food material for addition to some kinds of food and utilized in the field of specific health food. On the other hand, raffinose family oligosaccharides are neither digested nor absorbed in mammals such as human, but are assimilated and decomposed by enterobacteria to generate gases and to cause meteorism and absorption disorder. Therefore, it has been desired to appropriately regulate the amount of raffinose family oligosaccharides in food and feed.

Raffinose family oligosaccharides are synthesized by the raffinose family oligosaccharide biosynthesis system beginning with sucrose in many plants. This biosynthesis system normally involves a reaction for the sequential addition of galactosyl groups from galactinol through an α (1→6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule. Raffinose synthase is the enzyme concerned in the reaction for producing raffinose by allowing a D-galactosyl group derived from galactinol to form the α (1→6) bond with the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule in the first step of this biosynthesis system. It has been suggested that this enzyme constitutes a rate-limiting step in the above synthesis system, and therefore this enzyme is quite important in the control of biosynthesis of raffinose family oligosaccharides.

Then, a method for controlling an expression level or activity of raffinose synthase in plants by utilizing a raffinose synthase gene is effective to control a biosynthesis system of raffinose family oligosaccharides in plants to increase or decrease the production of raffinose in plants. Thus, a raffinose synthase gene which can be used in such a method has been desired.

OBJECTS OF THE INVENTION

The main object of the present invention is to provide novel raffinose synthase genes from plants.

This object as well as other objects and advantage of the present invention will become apparent to those skilled in the art from the following description.

SUMMARY OF THE INVENTION

Under these circumstances, the present inventors have intensively studied and succeeded in isolating novel genes encoding raffinose synthase from various plants. Thus, the present invention has been completed.

That is, the present invention provides:

1. A raffinose synthase gene which comprises a nucleotide sequence hybridizable with a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1,

(b) the nucleotide sequence represented by SEQ ID NO: 2,

(c) a nucleotide sequence encoding the amino acid sequence of represented by SEQ ID NO: 3,

(d) the nucleotide sequence represented by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4,

(e) a nucleotide sequence encoding the amino acid

sequence represented by SEQ ID NO: 5,

(f) the nucleotide sequence represented by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6,

(g) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7, and

(h) the nucleotide sequence represented by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8,

under stringent conditions, and encoding a protein being capable of binding D-galactosyl group through α (1→6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule to form raffinose.

2. A raffinose synthase gene comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1.

3. A raffinose synthase gene comprising the nucleotide sequence represented by SEQ ID NO: 2.

4. A raffinose synthase gene comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 3.

5. A raffinose synthase gene comprising the nucleotide sequence represented by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4.

6. A raffinose synthase gene comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5.

7. A raffinose synthase gene comprising the nucleotide sequence represented by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6.

8. A raffinose synthase gene comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7.

9. A raffinose synthase gene comprising the nucleotide sequence represented by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8.

10. A raffinose synthase gene comprising the nucleotide sequence represented by SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.

11. A nucleic acid comprising a partial nucleotide sequence of the raffinose synthase gene of any one of the above 1 to 10.

12. A method for detecting a nucleic acid containing a raffinose synthase gene which comprises detecting said nucleic acid by hybridization using the labeled nucleic acid of the above 11 as a probe.

13. A method for amplifying a nucleic acid containing a raffinose synthase gene which comprises amplifying

said nucleic acid by polymerase chain reaction (PCR) using the nucleic acid of the above 11 as a primer.

14. A method for obtaining a raffinose synthase gene which comprises the steps of:

detecting a nucleic acid containing said raffinose synthase gene by hybridization using the labeled nucleic acid of the above 11 as a probe, and

recovering the detected nucleic acid.

15. A method for obtaining a raffinose synthase gene which comprises the steps of:

amplifying a nucleic acid containing said raffinose synthase gene by PCR using the nucleic acid of the above 11 as a primer, and

recovering the amplified nucleic acid.

16. A nucleic acid comprising a nucleic acid containing the raffinose synthase gene of any one of the above 1 to 10 which is joined to a nucleic acid exhibiting promoter activity in a host cell.

17. A vector comprising the raffinose synthase gene of any one of the above 1 to 10.

18. A transformant, wherein the raffinose synthase gene of any one of the above 1 to 10 is introduced into a host cell.

19. A transformant, wherein the nucleic acid of the above 16 is introduced into a host cell.

20. A transformant, wherein the vector of the above 17 is introduced into a host cell.

21. The transformant of any one of the above 18 to 20, wherein the host is a microorganism.

22. The transformant of any one of the above 18 to 20, wherein the host is a plant.

23. A method for producing a raffinose synthase which comprises the steps of:

culturing or growing the transformant of any one of the above 18 to 22 to produce the raffinose synthase, and

collecting the raffinose synthase.

24. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 1.

25. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 3.

26. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 5.

27. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 7.

The term "nucleic acid" used herein means an oligomer compound or a high molecular compound which is generally called "DNA" or "RNA".

DETAILED DESCRIPTION OF THE INVENTION

The gene engineering techniques described below can

be carried out, for example, according to methods described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press, ISBN 0-87969-309-6; "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X; "Current Protocols In Protein Science" (1995), John Wiley & Sons, Inc. ISBN 0-471-11184-8.

The genes of the present invention can be obtained from soybean, plants belonging to the families Chenopdiaceae such as beet, etc. and Cruciferae such as mustard, rapeseed, etc. Specific examples of the genes of the present invention include those comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1, the nucleotide sequence represented by SEQ ID NO: 2, a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 3, the nucleotide sequence represented by SEQ ID NO: 4 or by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4, a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5, the nucleotide sequence represented by SEQ ID NO: 6 or by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6, a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7, the nucleotide sequence represented by SEQ ID NO: 8 or by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8, and the like.

The genes of the present invention can be obtained,

for example, by the following method.

That is, the genes of the present invention derived from soybean can be obtained, for example, by the following method.

For example, the gene can be obtained by a hybridization method using a nucleic acid having the nucleotide sequence represented by SEQ ID NO: 2 or its partial nucleotide sequence as a probe to detect a nucleic acid fragment which hybridizes to the probe in DNAs derived from soybean, followed by isolating the detected nucleic acid.

In this method, first, a nucleic acid to be used as the probe is prepared. As such a nucleic acid, for example, there is a nucleic acid composed of an oligonucleotide chemically synthesized by a conventional method on the basis of the nucleotide sequence of SEQ ID NO: 2. Specific example thereof includes a nucleic acid having the 800th to the 899th nucleotides in the nucleotide sequence represented by SEQ ID NO: 2.

Alternatively, the gene of the present invention derived from soybean can be obtained by the following method.

For example, tissue of soybean (*Glycine max*) is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From the tissue debris powder, RNA is extracted by a conventional method. A commercially available RNA extraction kit can be utilized in the extraction. RNA is recovered from thus-obtained RNA extract

by ethanol precipitation. Poly-A tailed RNA is fractionated from thus-recovered RNA by a conventional method. A commercially available oligo-dT column can be utilized in this fractionation. cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by using a commercially available cDNA synthesis kit. DNA is amplified by PCR using the above-obtained cDNA as the template and primers designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 2. More specifically, as the primers, for example, there are primers 11 and 12 shown in List 1 hereinafter. When PCR is carried out by using these primers and as the template cDNA derived from soybean, the genes of the present invention derived from soybean, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1" and the "raffinose synthase gene having the nucleotide sequence of SEQ ID No: 2" can be obtained.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X. Alternatively, cloning can be carried out, for example, by using a commercially available cloning kit such as TA cloning kit (Invitrogen) and a commercially available plasmid vector such

as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, pp. 5463-5467. For example, preferably, a commercially available kit such as ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit manufactured by Perkin-Elmer can be used.

List 1

Primer 11: (SEQ ID NO:9) ccaatctgat catgcttgtg ccgaa 25mer

Primer 12: (SEQ ID NO:10) ggaacaaagt tatgcactat tatttaaggt 30mer

The genes of the present invention derived from a Chenopdiaceae plant such as beet can be obtained by the following method.

For example, tissue of a Chenopdiaceae plant such as beet (*Beta vulgaris*) is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From this tissue debris powder, RNA is extracted by a conventional method. A commercially available RNA extraction kit can be utilized in the extraction. RNA is recovered from the thus-obtained RNA extract by ethanol precipitation. From the recovered RNA, poly-A tailed RNA is fractionated by a conventional method. A commercially available oligo-dT column can be utilized in this fractionation. cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by

utilizing a commercially available cDNA synthesis kit. DNA is amplified by PCR using the above-obtained cDNA as the template and primers designed and chemically synthesized on the basis of the nucleotide sequence of SEQ ID NO: 4. More specifically, as the primers, for example, there are primers 21 (SEQ ID NO:13) and 22 shown in List 2 hereinafter. When PCR is carried out by using these primers and as the template cDNA derived from beat, the genes of the present invention derived from beet, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 3," and the "raffinose synthase gene having a nucleotide sequence of SEQ ID No: 4" can be obtained. According to a particular purpose, the PCR primers can also be designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 4. For example, in order to amplify the "raffinose synthase gene having the nucleotide sequence represented by the 236th to the 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4", preferably, oligonucleotides having the nucleotide sequences represented by primers 23 (SEQ ID NO:14) and 24 (SEQ ID NO:15) in List 2 below are synthesized and used as the primers.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X.

Alternatively, cloning can be carried out by using a commercially available cloning kit such as TA cloning kit (Invitrogen) and a commercially available plasmid vector such as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined, for example, by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, pp. 5463-5467. For example, preferably, a commercially available kit such as ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit manufactured by Perkin-Elmer can be used.

List 2

(SEQ ID NO:11)
Primer 21: ctaccaaatt ccacaactta aagttca 27mer

(SEQ ID NO:12)
Primer 22: ggaataataa gcttcacaca tactgtactc tc 32mer

(SEQ ID NO:13)
Primer 23: atggctccaa gctttagcaa ggaaaattcc 30mer

(SEQ ID NO:14)
Primer 24: tcaaaataag tactcaacag tggtaaaacc 30mer

The genes of the present invention derived from Cruciferae plants such as mustard (*Brassica juncea*) and rapeseed (*Brassica napus*) can be obtained by the following method.

For example, tissue of a Cruciferae plant such as mustard or rapeseed is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From the tissue debris powder, RNA is extracted by a conventional method. A commercially available

RNA extraction kit can be utilized in the extraction. The RNA is recovered from thus-obtained RNA extract by ethanol precipitation. Poly-A tailed RNA is fractionated from the RNA thus recovered by a conventional method. A commercially available oligo-dT column can be utilized in the fractionation. cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by using a commercially available cDNA synthesis kit. DNA are amplified by PCR using the above-obtained cDNA as a template and primers designed and chemically synthesized on the basis of the nucleotide sequence of SEQ ID NO: 6. For example, when PCR is carried out by using cDNA derived from mustard (*Brassica juncea*) as the template and primers 33 and 34 (SEQ ID NO:33)/(SEQ ID NO:34) shown in List 3 hereinafter, the genes from Cruciferae plants of the present invention, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 5," and the "raffinose synthase gene having the nucleotide sequence represented by the 1st to 2654th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6" can be obtained. According to a particular purpose, the PCR primers can also be designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 6. For example, in order to amplify DNA encoding the open reading frame region of the "raffinose synthase gene having a nucleotide sequence encoding a protein having the

amino acid sequence of SEQ ID NO: 5", and the "raffinose synthase gene having the nucleotide sequence represented by the 134th to the 2467th nucleotides of SEQ ID NO: 6", preferably, oligonucleotides having the nucleotide sequences represented by primers 35 and 36 in List 3 are synthesized and used as the primers.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X. Alternatively, cloning can be carried out, for example, by using a commercially available TA cloning kit (Invitrogen) or a commercially available plasmid vector such as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, pp. 5463-5467. For example, preferably, the commercially available ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer can be used.

List 3

Primer 31: ttggaagaga agacgccgcc gggaatcgtc 30mer

Primer 32: ttaagccccg gcgagagctc tggccggaca 30mer

Primer 33: accaatccaa aatctcatca aataatcgca 30mer

Primer 34: (SEQ ID NO:18) aaataatagg ggcagtacaa attacaccac 30mer

Primer 35: (SEQ ID NO:19) atggctccac cgagcgtaat taaatccga 29mer

Primer 36: (SEQ ID NO:20) ctaaaactca tacttaatag aagacaaacc 30mer

Then, a nucleic acid having a partial nucleotide sequence of the gene of the present invention (hereinafter referred to as "the gene fragment") which is obtained by the above-described method is labeled and then used as a probe in a hybridization method. The probe can be hybridized to, for example, DNA derived from soybean, a Chenopdiaceae plant or a Cruciferae plant to detect a nucleic acid having the probe specifically bound thereto, thereby detecting a nucleic acid having the raffinose synthase gene.

As the DNA derived from soybean, a Chenopdiaceae plant such as beet or a Cruciferae plant such as mustard or rapeseed, for example, a cDNA library or a genomic DNA library of these plants can be used. The gene library may also be a commercially available gene library as such or a library constructed according to a conventional library construction method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X.

As the hybridization method, for example, plaque hybridization or colony hybridization can be employed, and they are selected depending upon the kind of vector used in the

construction of a library. More specifically, when the library to be used is constructed with a phage vector, a suitable host microorganism is mixed with the phage of the library under infectious conditions to obtain transformants. The transformant is further mixed with a soft agar medium, and the mixture is plated on an agar medium. Thereafter, the mixture is cultured at 37°C until a plaque of an appropriate size appears. When the library to be used is constructed with a plasmid vector, the plasmid is introduced into a suitable host microorganism to form transformants. The transformant obtained is diluted to a suitable concentration and the dilution is plated on an agar medium, after which it is cultured at 37°C until a colony of an appropriate size appears. In either case of the above libraries, a membrane filter is placed on the surface of the agar medium after the above cultivation, so that the phage or transformant is transferred to the membrane. This membrane is denatured with an alkali, followed by neutralization, and for example, when a nylon membrane is used, the membrane is irradiated with ultraviolet light, so that DNA of the phage or transformant is fixed on the membrane. This membrane is then subjected to a hybridization method wherein the gene fragment which has a partial nucleotide sequence of the gene of the present invention and labeled by a conventional method (hereinafter referred to as "the labeled gene fragment") is used as a probe. For this method, reference may be made, for example, to D.M.

Glover ed., "DNA cloning, a practical approach" IRL PRESS (1985), ISBN 0-947946-18-7. There are various reagents and temperature conditions to be used in the hybridization. For example, in general, prehybridization is carried out by immersion of the membrane in a prehybridization solution [6 x SSC (0.9 M NaCl, 0.09 M citric acid), 0.1 to 1 (w/v)% SDS, 100 µg/ml denatured salmon sperm DNA] and incubation at 65°C for 1 hour. Then, hybridization is carried out by addition and mixing of the labeled gene fragment thereto and incubating the membrane at 42 to 68°C for 4 to 16 hours.

In the present invention, the "stringent conditions" are those wherein incubation is carried out, for example, at 65 to 68°C in the above hybridization.

After hybridization, the membrane is taken out and is washed with 2 x SSC containing 0.1 to 1 (w/v)% SDS, further rinsed with 0.2 x SSC containing 0.1 to 1 (w/v)% SDS, and then dried. The membrane is analyzed, for example, by autoradiography or other techniques to detect the position of the probe on the membrane, thereby detecting the position on the membrane of a nucleic acid having a nucleotide sequence homologous to that of the probe used. The clone corresponding to the position of the nucleic acid thus detected on the membrane is identified on the original agar medium and the positive clone is selected so that the clone having the nucleic acid can be

isolated. The same procedures of detection are repeated to purify the clone having the nucleic acid.

Alternatively, a commercially available kit such as GENE TRAPPER cDNA Positive Section System kit (GibcoBRL) can be used. In this method, first, a single-stranded DNA library is hybridized with the biotynylated gene fragment (i.e., probe), followed by adding streptoavidin-bound magnet beads and mixing. From the mixture, the streptoavidin-bound magnetic beads are collected with a magnet, so that single-stranded DNA having a nucleotide sequence homologous to that of the probe used, which has been bound to these beads through the gene fragment, biotin and streptoavidin, is collected and detected. The single-stranded DNA collected can be converted into a double-strand form by reaction with a suitable DNA polymerase using a suitable oligonucleotide as a primer.

As described above, a nucleic acid containing raffinose synthase gene can obtained by detecting a nucleic acid hybridizable to the gene fragment in DNAs of a gene library derived from soybean, a Chenopdiaceae plant or a Cruciferae plant, purifying a clone having the nucleic acid and isolating phage or plasmid DNA from the clone. By preparing the restriction map or determining the nucleotide sequence of the nucleic acid thus obtained according to a conventional method, the nucleic acid containing the gene of the present invention can be confirmed.

For example, the gene of the present invention from

a Chenopdiaceae plant can be confirmed by the following point:

The amino acid encoded by the nucleotide sequence thus determined has 75% or more homology to the amino acid sequence represented by the 103rd to 208th amino acids in the amino acid sequence of SEQ ID NO: 3;

80% or more homology to the amino acid sequence represented by the 255th to 271st amino acids in the amino acid sequence of SEQ ID NO:3;

70% or more homology to the amino acid sequence represented by the 289th to 326th amino acids in the amino acid sequence of SEQ ID NO: 3; or

70% or more homology to the amino acid sequence represented by the 610th to 696th amino acids in the amino acid sequence of SEQ ID NO: 3.

The gene of the present invention from a Cruciferae plant can be confirmed, for example, by the following point:

The amino acid sequence encoded by the nucleotide sequence determined has 75% or more homology to the amino acid sequence represented by the 111th to 213th amino acids in the amino acid sequence of SEQ ID NO: 5;

80% or more homology to the amino acid sequence represented by the 260th to 275th amino acids in the amino acid sequence of SEQ ID NO: 5;

70% or more homology to the amino acid sequence represented by the 293rd to 325th amino acids in the amino acid

sequence of SEQ ID NO: 5; or

70% or more homology to the amino acid sequence represented by the 609th to 695th amino acids in the amino acid sequence of SEQ ID NO: 5.

The "homology" used herein means the proportion of the number of amino acids in a region, which are identical to those in a different region to be compared, to the number of the entire amino acids in the former region, upon comparing regions having similarity in two amino acid sequences. In this respect, it is preferred that the region having similarity contains more amino acids. Such homology of amino acid sequences can be evaluated by using a commercially available gene analysis software such as GENETIX (Software Kaihatu K.K.).

Further, according to the same manner as described above, a nucleic acid containing raffinose synthase gene can be detected by hybridization to DNA from the desired organism using the gene fragment as a probe to detect a nucleic acid to which the probe specifically binds (hereinafter referred to as the detection method of the present invention). The gene fragment used herein can be chemically synthesized according to a conventional method on the basis of the nucleotide sequence represented by SEQ ID NO: 2, 4, 6 or 8. Alternatively, it can be prepared by PCR using as primers oligonucleotides chemically synthesized according to a conventional method on the basis of the nucleotide sequence represented by SEQ ID NO: 2, 4, 6 or

8.

The gene fragment may be a part of the non-translated region of the raffinose synthase gene as well as the open reading frame thereof. For example, an oligonucleotide having the same nucleotide sequence as a part of that of 5'-upstream side such as the 1st to 235th nucleotides in the nucleotide sequence of SEQ ID NO: 4, the 1st to 133rd nucleotides in the nucleotide sequence of SEQ ID NO: 6 and the like, or a part of that of 3'-downstream side such as the 2588th to 2675th nucleotides in the nucleotide sequence of SEQ ID NO: 4, the 2468th to 2676th nucleotides in the nucleotide sequence of SEQ ID NO: 6 and the like.

When PCR is carried out by using the gene fragment as primers, it is possible to amplify a nucleic acid containing raffinose synthase gene from DNA derived from the desired organism (hereinafter referred to as the amplification method of the present invention).

More specifically, for example, oligonucleotides having the nucleotide sequences of the gene fragment are designed and chemically synthesized according to a conventional method. In general, it is preferred that the number of nucleotides is more from a viewpoint that the specificity of annealing is ensured. It is, however, also preferred that the number of nucleotides is not so many from viewpoints that the primers themselves are liable to have a higher structure giving possible

deterioration of the annealing efficiency and that complicated procedures are required in the purification after the synthesis. Normally, oligonucleotides composed of 15 to 50 bases are preferred. In this respect, based on the codon table showing the correspondence of amino acids encoded by codons, a mixture of primers can also be synthesized by using a mixture of plural bases so that a residue at a specified position in a primer is changed to different bases according to the variation of codons which can encode one certain amino acid. Alternatively, for example, a base such as inosine which can form a base pair with plural bases can be used instead of the above mixture of plural bases.

Coding Table

Phe: UUU, UUC Ser: UCU, UCC, UCA, UCG, AGU, AGC

Tyr: UAU, UAC Cys: UGU, UGC

Stop: UAA, UAG, UGA Trp: UGG

Leu: UUA, UUG, CUU, CUC, CUA, CUG Pro: CCU, CCC, CCA, CCG His: CAU, CAC Gln: CAA, CAG Arg: CGU, CGC, CGA, CGG, AGA, AGG

Ile: AUU, AUC, AUA Thr: ACU, ACC, ACA, ACG

Asn: AAU, AAC Lys: AAA, AAG

Met: AUG

Val: GUU, GUC, GUA, GUG Ala: GCU, GCC, GCA, GCG

Asp: GAU, GAC Gly: GGU, GGC, GGA, GGG

Glu: GAA, GAG

In the above codon table, each codon is shown as the nucleotide sequence in mRNA and its light hand is the 5'-terminus. U represents uracil base in RNA and corresponds to thymine base in DNA.

An oligonucleotide having the same nucleotide sequence as the coding strand of the double-stranded DNA of the gene of the present invention is called a "sense primer" and that having a nucleotide sequence complementary to the coding strand is called an "antisense primer".

A sense primer having the same nucleotide sequence as that of 5'-upstream side in the coding strand of the gene of the present invention, and an antisense primer having a nucleotide sequence complementary to the nucleotide sequence on the 3'-downstream side in this coding strand are used in combination for PCR reaction, for example, with a gene library, genomic DNA or cDNA as the template to amplify DNA. As the gene library to be used, for example, there are a cDNA library and a genomic library derived from soybean, a Chenopdiaceae plant such as beet or a Cruciferae plant such as mustard or rapeseed, etc. The gene library may also be a library constructed according to a conventional library construction method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X, or a commercially available gene

library as such. As the genomic DNA or cDNA, for example, there are those prepared from soybean, a Chenopdiaceae plant such as beet or a Cruciferae plant such as mustard or rapeseed, etc. For example, PCR is carried out by using the primers 31 (SEQ ID NO: 15) and 32 (SEQ ID NO: 16) in the above List 3 and as the template cDNA derived from mustard to amplify DNA having the nucleotide sequence represented by the 749th to 1215th nucleotides in the nucleotide sequence of SEQ ID NO: 6. Further, PCR is carried out by using the primers and as the template cDNA derived from rapeseed to amplify DNA having the nucleotide sequence represented by the 1st to 467th nucleotides in the nucleotide sequence of SEQ ID NO: 8. The nucleic acid thus amplified can be confirmed by conventional electrophoresis. The nucleic acid can be cloned according a conventional method such as that described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor laboratory Press or "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Inc., ISBN 0-471-50338-X. For the nucleic acid, its restriction map is prepared or its nucleotide sequence is determined by a conventional method, so that the nucleic acid containing raffinose synthase gene or a part thereof can be identified. When the nucleic acid contains a part of raffinose synthase, PCR can be carried out on the basis of its nucleotide sequence to amplify the nucleic acid containing the 5'-upstream side nucleotide sequence or the 3'-downstream side nucleotide

sequence. That is, based on the nucleotide sequence of the above-obtained nucleic acid, an antisense primer is designed and synthesized for amplification of the 5'-upstream side part, and a sense primer is designed and synthesized for amplification of the 3'-downstream side part. The nucleotide sequence of the 5'-upstream side part or 3'-downsream side part of the nucleotide sequence already obtained can be determined by the RACE method using these primers and a commercially available kit such as Marathon Kit of Clontech. The full length raffinose synthase gene can be obtained by synthesizing new primers based on both terminal sequences in the nucleotide sequence thus determined and carrying out PCR again.

The above detection method of the present invention can also be used in the analysis of genotypes of a plant such as soybean, a Chenopdiaceae plant or a Cruciferae plant, etc. More specifically, for example, a genomic DNA derived from soybean, a Chenopdiaceae plant or a Cruciferae plant is prepared according to a conventional method, for example, described in "Cloning and Sequence (Plant Biotechnology Experiment Manual)" complied under the supervision of Itaru Watanabe, edited by Masahiro Sugiura, published by Noson Bunka-sha, Tokyo (1989). The genomic DNA is digested with at least several kinds of restriction enzymes, followed by electrophoresis. The electrophoresed DNA is blotted on a filter according to a conventional method. This filter is subjected to hybridization

with a probe prepared from DNA having the gene fragment by a conventional method, and DNA to which the probe hybridizes is detected. The DNAs detected are compared in length between different varieties of a specified plant species. The differences in length make possible the analysis of differences in phenotypic characteristics accompanied with the expression of raffinose family oligosaccharides between these varieties. Furthermore, when the DNAs detected by the above method are compared in length between the gene recombinant plant and the non-gene recombinant plant of the same variety, the former plant can be distinguished from the latter plant by the detection of hybridizing bands greater in number or higher in concentration for the former plant than for the latter plant. This method can be carried out according to the RFLP (restriction fragment length polymorphism) method, for example, described in "Plant PCR Experiment Protocols" complied under the supervision of Ko Shimamoto and Takuji Sasaki, published by Shujun-sha, Tokyo (1995), ISBN 4-87962-144-7, pp. 90-94.

Further, the amplification method of the present invention can be used for an analysis of genes of soybean, a Chenopdiaceae plant or a Cruciferae plant, etc. More specifically, for example, the amplification method of the present invention is carried out by using plant genomic DNA prepared from soybean, a Chenopdiaceae plant or a Cruciferae plant to amplify DNA. The amplified DNA is mixed with a

formaldehyde solution, followed by heat denaturing at 85°C for 5 minutes and then quickly cooling on ice. A sample thereof is subjected to electrophoresis on, for example, 6 (w/v)% polyacrylamide gel containing 0 (v/v)% or 10 (v/v)% of glycerol. For this electrophoresis, a commercially available electrophoresis apparatus such as that for SSCP (Single Strand Conformation Polymorhism) can be used and the electrophoresis can be carried out with maintaining the gel at a constant temperature, for example, at 5°C, 25°C, 37°C, etc. From the electrophoresed gel, DNA is detected, for example, by a method such as silver staining method with a commercially available reagent. From the differences of behavior between the varieties in the electrophoresis of the DNA detected, a mutation in the raffinose synthase gene is detected, and an analysis is carried out for differences caused by the mutation in phenotypic characteristics accompanied with the expression of raffinose family oligosaccharides. This method can be carried out according to the SSCP method, for example, described in "Plant PCR Experiment Protocols" complied under the supervision of Ko Shimamoto and Takuji Sasaki, published by Shujun-sha, Tokyo (1995), ISBN 4-87962-144-7, pp. 141-146.

The analysis of the plant gene from soybean, a Chenopdiaceae plant or a Cruciferae plant by the above detection method or amplification method of the present invention can be used not only for the analysis of differences in phenotypic

characteristics accompanied with the expression of raffinose family oligosaccharides, but also, for example, for the selection of clones having the desired characters upon production of a novel variety of soybean, a Chenopdiaceae plant or a Cruciferae plant. Further, it can also be used for identification of a clone thus produced and having the characters derived from a recombinant plant upon producing a plant variety using the recombinant plant.

For expression of the gene of the present invention in cells of a host, preferably, a nucleic acid comprising a nucleic acid fragment which contains the gene of the present invention, and a nucleic acid fragment which has a promoter activity in the host cells and joined to the former nucleic acid fragment (hereinafter referred to as the expression nucleic acid of the present invention) can be used.

The nucleic acid fragment having promoter activity in the expression nucleic acid of the present invention is not limited to a specific one, so long as it is functionable in a host to be transformed. For example, there are synthetic promoters functionable in *Escherichia coli*, such as *E. coli* lactose operon promoter, *E. coli* tryptophan operon promoter and tac promoter, etc.; yeast alcohol dehydrogenase gene (ADH) promoter, adenovirus major late (Ad.ML) promoter, SV40 early promoter, baculovirus promoter and the like. When the host is

a plant, the promoter may include, for example, T-DNA derived constitutive promoters such as nopaline synthase gene (NOS) promoter, octopine synthase gene (OCS) promoter, etc.; plant virus-derived promoters such as cauliflower mosaic virus (CaMV)-derived 19S and 35S promoters; inducible promoters such as phenylalanine ammonia-lyase (PAL) gene promoter, chalcone synthase (CHS) gene promoter, pathogenesis-related protein (PR) gene promoter, etc. Furthermore, vector pSUM-GY1 (see JP-A 06-189777/1994) can also be used, which has a promoter giving specific expression in a specified plant tissue, e.g., a promoter of soybean-derived seed storage protein glycinin gene (JP-A 6-189777).

Furthermore, a nucleic acid fragment having a terminator activity can be joined to the expression nucleic acid of the present invention. In this case, it is generally preferred that the expression nucleic acid of the present invention is constructed so that the nucleic acid fragment having a terminator activity is positioned downstream the raffinose synthase gene. The terminator to be used is not particularly limited, so long as it is functionable in cells of a host to be transformed. For example, when the host is a plant, there are T-DNA derived constitutive terminators such as nopaline synthase gene (NOS) terminator, etc.; plant derived terminators such as terminators of allium virus GV1 or GV2, and the like.

The expression nucleic acid of the present invention

can be introduced into a host cell according to a conventional gene engineering technique to obtain a transformant. If necessary, the expression nucleic acid of the present invention can be inserted into a vector having a suitable marker depending upon a particular transformation technique for introduction of the nucleic acid into a host cell.

A vector into which the expression nucleic acid of the present invention is inserted can be introduced into a microorganism according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor laboratory Press or "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Inc., ISBN 0-471-50338-X. The microorganism transformed with the vector can be selected on the basis of a selection marker such as antibiotic resistance, auxotrophy or the like. In case that the gene of the present invention is joined to the downstream of an inducible promoter (e.g., tac promoter) in the translatable form in the selected microorganism (e.g., *E. coli* transformant), a translated product of the gene of the present invention can be expressed under conventional culture and inducible conditions and can be recover as a peptide or a protein.

The raffinose synthase activity of the translated product of the gene of the present invention thus prepared can be measured by, for example, a method described in L. Lehle and W. Tanner, Eur. J. Biochem., 38, 103-110 (1973) to identify the

translated product having the "capability of binding D-galactosyl group through α (1→6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in sucrose molecule". More specifically, for example, the gene of the present invention is cloned in pGEX-4T3 (Pharmacia) to obtain a plasmid containing the expression nucleic acid of the present invention. The resultant plasmid is introduced into, for example, *E. coli* HB101 strain to obtain a transformant. The resultant transformant is culture overnight and 1 ml of the culture is inoculated into 100 ml of LB culture medium. It is incubated at 37°C for about 3 hours and IPTG (isopropylthio-β-D-galactoside) is added at a final concentration of 1 mM, followed by further incubation for 5 hours. Cells are recovered from the culture broth by centrifugation and are suspended by addition of 10 times of the cell weight of 100 mM Tris-HCl (pH 7.4), 1 mM EDTA, 5 mM DTT (dithiothreitol), 1 mM PMSF (phenylmethylsulfonyl fluoride) and 1 mM benzamide. The suspension is sonicated with a ultrasonic disrupter (Branson) to disrupt the cells. The disrupted cell suspension is centrifuged to recover a soluble protein solution. The resultant protein solution is added to a reaction mixture containing at final concentrations of 100 mM Tris-HCl (pH 7.4), 5 mM DTT (dithiothreitol), 0.01% BSA, 200 μM sucrose, 5 mM galactinol and 31.7 μM [^{14}C] sucrose. The reaction mixture is incubated at 37°C, followed by addition of 1.5 times in volume

of ethanol and stirring. Insoluble materials are remove by centrifugation, the supernatant is spotted on, for example, a HPTLC cellulose thin layer chromatography plate (Merch, HPTLC plates cellulose) and then the plate is developed with n-butanol-pyridine-water-acetic acid (60 : 40 : 30 : 3). The developed plate is dried and analyzed with an imaging analyzer (FUJIX Bio-Image Analyzer BAS-2000II manufactured by Fuji Film) to determine [^{14}C] raffinose produced to measure the raffinose synthase activity.

In addition, the translated product as prepared above can also be used as an antigen for producing an antibody. The antibody thus produced can be used for, for example, detection and determination of the gene of the present invention in a crude protein extract prepared from an organism such as a plant.

When the host is a plant, the vector into which the gene of the present invention is inserted can be introduced into plant cells by a conventional means such as *Agrobacterium* infection method (JP-B 2-58917 and JP-A 60-70080), electroporation into protoplasts (JP-A 60-251887 and JP-B 5-68575) or particle gun method (JP-A 5-508316 and JP-A 63-258525). The plant cell transformed by the introduction of the vector can be selected on the basis of a selection marker, for example, resistance to an antibiotic such as kanamycin or hygromycin. From the plant cell thus transformed, a transformed plant can be regenerated by a conventional plant cell cultivation

method, for example, described in "Plant Gene Manipulation Manual (How to Produce Transgenic Plants)" written by Uchimiya, 1990, Kodan-sha Scientific (ISBN 4-06-153513-7), pp. 27-55. Furthermore, the collection of seeds from the transformed plant also makes it possible to prolify the transformed plant. In addition, crossing between the transformed plant obtained and the non-transformed plant makes it possible to produce progenic plants with the characters of the transformed plant.

As gene engineering techniques in soybean, basically, the above general techniques can be employed. More specifically, "transformation of soybean plant strain by particle gun" described in EP 301749, gene introduction methods. for example, described in Torisky, R.S., Kovacs, L., Avdiushko, S., Newman, J.D., Hunt, A.G. and Collins, G.B., "Development of a binary vector system for plant transformation based on the supervirulent Agrobacterium tumefaciens strain Chry5", Plant Cell Rep., (1997), 17, p. 102-108, etc. can be employed.

As gene engineering techniques in a Chenopdiaceae plant, basically, the above general techniques can be employed. More specifically, gene introduction methods, for example, described in M. Mannerlof, S. Tuvesson, P. Steen and P. Tenning, "Transgenic sugar beet tolerant to glyphosate", Euphytica (1997), 94, p 83-91, B.K. Konwar, "Agrobacterium tumefacience-Mediated Genetic Transformation of Sugar Beet (Beta vulgaris L.)", J. Plant Biochemistry & Biotechnology

(1994), 3, p. 37-41 can be employed.

As gene engineering techniques in a Cruciferae plant, basically, the above general techniques can be employed. More specifically, the gene introduction can be carried out according to a method, for example, described in J. Fry, A. Barnason and R.B. Horsch, "Transformation of Brassica napus with Agrobacterium tumefaciens based vectors", Plant Cell Reports (1987), 6, 321-325.

For example, when gene introduction is carried out by *Agrobacterium* infection method, first, the above-described expression nucleic acid of the present invention is inserted into a binary vector. The resultant vector can be introduced into, for example, *Agrobacterium tumefaciens* LBA 4404 strain which has been converted into a competent state by treatment with calcium chloride. A transformant can be selected by an appropriate selection method according to the selection marker gene of the vector, for example, cultivation of a strain containing the vector in a culture medium containing an antibiotic in case that the selection marker gene is that giving resistance to the antibiotic such as kanamycin. The resultant transformed *Agrobacterium* strain can be culture in a liquid culture medium, for example, LB medium.

Soybean, a Chenopdiaceae plant or a Cruciferae plant can be transformed by using thus obtained *Agrobacterium*

transformant culture broth as described below. For example, seeds from soybean, beet, rapeseed or mustard is sowed aseptically in, for example, 1/2 MS medium containing 2% sucrose and 0.7% agar. After about 1 week, cotyledons and petioles of the germinated plant are cut off with a scalpel aseptically and transplanted in, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5 μM BA, 0.05 μM 2,4-D and 3.3 μM $AgNO_3$ and cultured for one day. The cotyledons and petioles thus precultured are transferred to 1000-fold dilution of the above *Agrobacterium* culture broth and allowed to stand for 5 minutes. The cotyledons and petioles are transferred to the same medium as that of the preculture again and cultured for about 3 to 4 days. The cotyledones and petioles thus cultured are transferred to, for example, MS medium containing 3% sucrose, 4.5 μM BA, 0.05 μM 2,4-D, 3.3 μM $AgNO_3$ and 500 mg/liter cefotaxim, followed by shaking for 1 day to remove microbial cells. The resultant cotyledons and petioles are transferred to, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5 μM BA, 0.05 μM 2,4-D, 3.3 μM $AgNO_3$ 100 mg/liter cefotaxim and 20 mg/liter kanamycin, followed by culturing for 3 to 4 weeks. Then, the cotyledons and petioles are transferred to, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5 μM BA, 0.05 μM 2,4-D, 100 mg/liter cefotaxim and 20 mg/liter kanamycin and cultured. Culture in this medium is continued with subculturing every 3 to 4 weeks. When a shoot are regenerated, it is subcultured in,

for example, MS medium containing 3% sucrose, 0.7% agar and 20 mg/liter kanamycin for 3 to 4 weeks. When the plant makes roots, it is transferred to vermiculite-peat moss (1 : 1) and acclimatized by culturing at 21 to 22°C under day and night conditions of 12 hours: 12 hours = day time : night. As the plant grows, it is transferred to appropriate cultivation soil to culture the plant. A genomic DNA is extracted from the leaf of the regenerated plant according to the above method and PCR is carried out by using as primers having partial nucleotide sequences of the expression nucleic acid of the present invention to confirm the insertion of the gene of the present invention into the plant.

As described hereinabove, by introducing the gene of the present invention into a plant, for example, soybean, a Chenopdiaceae plant or a Cruciferae plant, it is possible to vary the expression level and activity of raffinose synthase in the plant to control the amount of raffinose family oligosaccharides in the plant. The gene of the present invention is useful in techniques for varying the expression level and activity of raffinose synthase in soybean, a Chenopdiaceae plant or a Cruciferae plant on the basis of gene homology, for example, techniques such as homologous recombination and antisense technique, cosuppression and the like.

The following examples further illustrate the present

invention in detail but are not to be construed to limit the scope of the present invention.

Example 1

Preparation of cDNA Derived from Soybean

About 2 g of immature seeds of soybean (*Glycine max*) Williams82 were frozen in liquid nitrogen and then ground with a mortar, to which 20 ml of Isogen (Nippon Gene) was added, and the mixture was further thoroughly ground. The ground material was transferred into a centrifugation tube, to which 4 ml of chloroform was added, and the mixture was stirred with a vortex mixer and then centrifuged at 6,500 x g for 10 minutes at 4°C. The water layer was collected, to which 10 ml of isopropanol was added, and the mixture was stirred and then centrifuged at 6,500 x g for 10 minutes at 4°C. The resulting precipitate was washed with 10 ml of 70% ethanol and then dissolved in 1 ml of elution buffer (10 mM Tris-HCl/pH 7.5, 1 mM EDTA, 0.1% SDS). The solution was allowed to stand at 60°C for 10 minutes and then centrifuged at 10,000 x g for 1 minute to remove insoluble matter. To the resulting supernatant was added an equivalent volume of Oligotex-dT30 (Takara), and the mixture was stirred and then allowed to stand at 65°C for 5 minutes. Further, the mixture was placed on ice and allowed to stand for 3 minutes, to which 200 µl of 5 M NaCl was added, and the mixture was mixed and then allowed to stand at 37°C for 10 minutes. The mixture was then centrifuged

at 10,000 x g for 3 minutes at 4°C. The precipitate was collected and then suspended in 1 ml of TE buffer, and the suspension was allowed to stand at 65°C for 5 minutes. Further, the suspension was placed on ice and then allowing to stand for 3 minutes, followed by centrifugation at 10,000 x g for 3 minutes at 4°C to remove precipitate.

To the resulting supernatant were added 100 µl of 3M sodium acetate and 2 ml of ethanol to precipitate and collect RNA. The collected RNA was washed twice with 70% ethanol and then dissolved in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

For the cDNA synthesis, First Strand Synthesis Kit for RT-PCR (Amersham) and cDNA Synthesis Kit (Takara) were used, and all operations were made according to the protocol attached to kits.

Example 2

Cloning of Raffinose Synthase Gene from Soybean cDNA

PCR was carried out by using the cDNA obtained from immature seeds of soybean (*Glycine max*) Williams82 in Example 1 as a template and the primers designed on the basis of the amino acid sequence of SEQ ID No: 1, i.e., primers having nucleotide sequences shown in List 4 (SEQ ID NOS: 21+22) below to amplify a DNA fragment. The PCR was carried out with Gene Amp PCR Systems 2400 and DNA Thermal

Cycler Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech. The reaction was carried out by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times to amplify the DNA fragment. The amplified DNA fragment was cloned with TA cloning kit (Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and analysis of the nucleotide sequence with a 373S DNA sequencer of ABI. Based on this sequence, primers having nucleotide sequences shown in List 5 (SEQ ID NO:23) below were synthesized. The synthesis of cDNA was carried out with Marathon Kit of Clontech using mRNA obtained from leaves of soybean Williams82 in Example 1. The cDNA obtained was ligated to an adapter contained in the kit with ligase. These operations were carried out according to the protocol attached to the kit. By using the adapter-ligated cDNA thus prepared, PCR was carried out with the primers shown in List 5 (SEQ ID NO) according to the same manner as the above. The nucleotide sequence in terminal region of the gene was analyzed according to the protocol attached to the Marathon Kit of Clontech. As a result, the nucleotide sequence of SEQ ID NO: 2 was determined.

List 4

4-5-F primer: (SEQ ID NO:21)

cgatggatgg giaaittiat icaiccigai tgggaiatgt t 41mer

4-6-RV primer: (SEQ ID NO:22)

ggccacatit tiacia(ag)icc iatiggigci aa 32mer

List 5

5-SC-2: (SEQ ID NO:23)

tgttactagg cgaaacaaga gtagctctga 30mer

Example 3

Preparation of cDNA derived from Chenopdiaceae Plant

About 2 g of leaves of beet (*Beta vulgaris*: haming) was frozen in liquid nitrogen and then ground with a mortar, to which 20 ml of Isogen (Nippon Gene) was added, and the mixture was further thoroughly ground. The ground material was transferred into a centrifugation tube, to which 4 ml of chloroform was added, and the mixture was stirred with a vortex mixer and then centrifuged at 6,500 x g for 10 minutes at 4°C. The water layer was collected, to which 10 ml of isopropanol was added, and the mixture was stirred and then centrifuged at 6,500 x g for 10 minutes at 4°C. The resulting precipitate was washed with 10 ml of 70% ethanol and then dissolved in 180 µl of DEPC-treated sterilized water. The solution was allowed to stand at 55°C for 5 minutes and 20 µl of 5 M NaCl was added thereto. The resulting solution was purified using BIOMAG mRNA PURIFICATION KIT (PerSeptive Biosystems: Catalog No. 8-MB4003K).

To the resulting mRNA solution were added 3M sodium acetate and ethanol, and RNA was precipitated and collected. The collected RNA was washed twice with 70% ethanol and then dissolved

in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

For the cDNA synthesis, SMART PCR cDNA Synthesis Kit (Clontech) was used, and all operations were made according to the protocol attached to the kit.

Example 4

Analysis of Nucleotide Sequence of Raffinose Synthase Gene from Chenopdiaceae Plant

Synthetic DNA primers having the nucleotide sequences shown in List 6 (SEQ ID NOS: 24-27) below were synthesized. The PCR method was carried out with Gene Amp PCR Systems 2400 and DNA Thermal Cycler Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech. The PCR was carried out with the above primers (SEQ ID NOS: 24-27) and cDNA of beet obtained in the above Example 3 by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times. As a result, the combinations of primers 6-3-F (SEQ ID NO: 24) and 6-8-RV (SEQ ID NO: 25) and primers 6-10-F (SEQ ID NO: 26) and 6-6-RV (SEQ ID NO: 27) gave an amplification of about 0.3 kb and 0.6 kb bands, respectively. The amplified DNA fragments were cloned with TA cloning kit (Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and analysis of nucleotide sequence with a 373S DNA sequencer of ABI. Based on the resulting nucleotide sequences, synthetic DNA

(SEQ ID NOS: 28-35)

primers having nucleotide sequences shown in List 7 below were prepared and PCR was carried out using cDNA obtained from beet in Example 3 in the same manner as above. As a result, DNA having the nucleotide sequence of SEQ ID NO: 4 was finally obtained from cDNA of beet.

List 6

6-3-F: (SEQ ID NO: 24)

cgaggiggit gicciccigg ittigtiati atigaigaig gitggca 47mer

6-8-RV: (SEQ ID NO: 25)

at(t/c)tt(a/g)tcia cigcia(a/g)(a/g)tc (t/c)tccatigt 29mer

6-10-F: (SEQ ID NO: 26)

ggiacitait gg(c/t)ticaigg itgicaiatg gticaitg 38mer

6-6-RV: (SEQ ID NO: 27)

ggccacatit tiacia(a/g)icc iatiggigci aa 32mer

List 7

7-Sb-1: (SEQ ID NO: 28)

atctatttgt catgacgatg atccga 26mer

7-Sb-2RV: (SEQ ID NO: 29)

ggccctcatt cccatattgg gatgatcctc 30mer

7-Sb-3RV: (SEQ ID NO: 30)

aagcatgcca aacatacaca tgctcaacag 30mer

7-Sb-4RV: (SEQ ID NO: 31)

agacccgggg aaagctttgg ggttactact 30mer

7-Sb-5: (SEQ ID NO: 32)

tggatgggaa actttataca ccctgact 28mer

7-Sb-6: (SEQ ID NO:33)

gacatgttcc catctacaca cccttgtg 28mer

7-Sb-7: (SEQ ID NO:34)

ccaatttatg ttagtgatgt tgttggcaag 30mer

7-Sb-8RV: (SEQ ID NO:35)

tcgactccca gggtagaatt gtcatc 26mer

Example 5

Preparation of cDNA Derived from Cruciferae Plant

About 2 g of leaves of mustard (*Brassica juncea*) was frozen in liquid nitrogen and then ground with a mortar, to which 20 ml of Isogen (Nippon Gene) was added, and the mixture was further thoroughly ground. The ground material was transferred into a centrifugation tube, to which 4 ml of chloroform was added, and the mixture was stirred with a vortex mixer and then centrifuged at 6,500 x g for 10 minutes at 4°C. The water layer was collected, to which 10 ml of isopropanol was added, and the mixture was stirred and then centrifuged at 6,500 x g for 10 minutes at 4°C. The resulting precipitate was washed with 10 ml of 70% ethanol and then dissolved in 180 µl of DEPC-treated sterilized water. The solution was allowed to stand at 55°C for 5 minutes and to which 20 µl of 5 M NaCl was added. The resulting solution was purified using BIOMAG mRNA PURIFICATION KIT (PerSeptive Biosystems: Catalog No. 8-MB4003K).

To the resulting mRNA solution were added 3M sodium

acetate and ethanol, and RNA was precipitated and collected. The collected RNA was washed twice with 70% ethanol and then dissolved in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

For the cDNA synthesis, SMART PCR cDNA Synthesis Kit (Clontech) was used, and all operations were carried out according to the protocol attached to the kit.

In the same manner as described in the above, mRNA was purified from immature seeds of rapeseed Westar (*Brassica napus*) and cDNA was synthesized.

Example 6

Isolation and Nucleotide Sequence Analysis of Raffinose Synthase Gene derived from Cruciferae Plant

DNA primers having the nucleotide sequences shown in List 8 (SEQ ID NOS:36+37) below were synthesized. PCR was carried out with Gene Amp PCR Systems 2400 and DNA Thermal Cycler Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech. The PCR was carried out with the above primers (SEQ ID NOS:36+37) and cDNA of mustard obtained in Example 5 by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times. The reaction products were analyzed by agarose gel electrophoresis. As a result, an amplification of about the 1.2 kb bands was detected. The amplified DNA fragment was cloned with TA cloning kit

(Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and nucleotide sequence analysis with a 373S DNA sequencer of ABI. Based on the resulting nucleotide sequence, synthetic primers having the nucleotide sequences shown in List 9 (SEQ ID NOS: 38-39) below were prepared and PCR was carried out using cDNAs from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica napus*) obtained in Example 5 according to the same manner as the above. As a result, the nucleotide sequence represented by the 749th to 1215th nucleotides of SEQ ID NO: 6 and by the 1st to 467th nucleotides of SEQ ID NO: 8 were finally determined for cDNA from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica napus*), respectively.

List 8

8-#1: (SEQ ID NO: 36)

cgattiaaig titggtggac iacicaitgg gtigg 35mer

8-#10RV: (SEQ ID NO: 37)

caitgiacca titgicaicc itgia(ag)ccai taigticc 38mer

List 9

9-primer-1: (SEQ ID NO: 38)

gttagggttc atatgaacac cttcaagctc 30mer

9-primer-2RV: (SEQ ID NO: 39)

caacggcgag atcttgcatc gtcaac 26mer

Example 7

Nucleotide Sequence Analysis of Raffinose Synthase

Full-Length Gene Derived from Cruciferae Plant

Based on the nucleotide sequences obtained in Example 6, (SEQ ID NOS: 6, 8, 36-39) DNA primers having the nucleotide sequences shown in List 10 below were synthesized. (SEQ ID NOS: 40-46) The cDNAs from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica napus*) obtained in the same manner as described in Example 5 were ligated to adapters contained in Marathon Kit of Clontech. By using the adapter-ligated cDNAs thus prepared, PCR was carried out with primers shown in List 10. 10-B-2RV, (SEQ ID NO: 40) 10-B-3RV (SEQ ID NO: 41) and 10-B-4RV primers were used for nucleotide analysis of 5'-termini, and 10-B-1, (SEQ ID NO: 43) 10-B-8, (SEQ ID NO: 45) 10-B-7 and 10-B-6 (SEQ ID NO: 46) primers were used for nucleotide analysis of 3'-termini. The nucleotide sequences were analyzed according to the protocol attached to the Marathon Kit of clontech. As a result, the nucleotide sequence of SEQ ID NO: 6 and SEQ ID NO: 8 were determined from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica napus*), respectively.

List 10

10-B-2RV: (SEQ ID NO: 40)

ggattcgaca caaaccgcca cgtcatcgtc 30mer

10-B-3RV: (SEQ ID NO: 41)

ccacgtgcac cacccgaact tatcgac 27mer

10-B-4RV: (SEQ ID NO: 42)

aacatcgata ccatcggagt catgtccaat 30mer

10-B-1: (SEQ ID NO: 43

gttagggttc atatgaacac cttcaagctc 30mer

10-B-8: (SEQ ID NO: 44)

tctacgtctg gcacgcgctt tgcggctac 29mer

10-B-7: (SEQ ID NO: 45)

gttgacgtca tccacatatt ggagatgttg t 31mer

10-B-6: (SEQ ID NO: 46)

gttatcgcta gcatggagca ctgtaatga 29mer

Example 8

Construction of Expression Vectors in Plant for Raffinose Synthase Gene Derived from Cruciferae Plant

Based on the nucleotide sequence of raffinose synthase gene from mustard obtained in Example 7, DNA primers having the nucleotide sequences shown in List 11 (SEQ ID NOS: 47-48) were prepared. By using cDNA of mustard, PCR was carried out in the same manner as described in Example 6. The amplified DNA fragment was digested with SacI. The DNA fragment thus digested was ligated to the vector pBI121(-) previously digested with SacI by using Ligation Kit (Takara). Plasmid pBI121 (Clontech) were digested with BamHI and SacI, and ligated to linkers shown in List 12 (SEQ ID NOS: 49-50) to prepare the vector pBI121(-). The vector thus obtained was analyzed by a restriction map and PCR using primers having nucleotide sequences shown in List 13, (SEQ ID NOS: 51-63) and confirmed the direction of inserted raffinose synthase gene. The vector whose raffinose synthase gene from mustard was inserted in the expressible direction was designated BjRS-Sac(+)-121 and the one whose

raffinose synthase gene from mustard was inserted in the reverse direction was designated BjRS-Sac(-)-121.

List 11

11-SacI-BjN:(SEQ ID NO:47)

aacgagctca atccaaaatc tcatcaaata atcgc 35mer

11-SacI-BjintRV:(SEQ ID NO:48)

acaatagttg agggcggaag agtag 25mer

List 12

12-BamSac-(+)linker:(SEQ ID NO:49)

gatcgagctc gtgtcggatc cagct 25mer

12-BamSac-(-)linker:(SEQ ID NO:50)

ggatccgaca cgagctc 17mer

List 13

13-35S-3:(SEQ IDNO:51)

cctcctcgga ttccattgcc cagctatctg 30mer

13-B-2RV:(SEQ ID NO:52)

ggattcgaca caaaccgcca cgtcatcgtc 30mer

13-B-8:(SEQ ID NO:53

tctacgtctg gcacgcgctt tgcggctac 29mer

Example 9

Transformation with Raffinose Synthase Gene Derived from Cruciferae Plant

The vectors BjRS-Sac(+)-121 and BjRS-Sac(-)-121 prepared in Example 8 were used for the transformation of mustard (*Brassica juncia*) by the *Agrobacterium* infection method.

Agrobacterium tumefacients (strain LBA4404 having rifampicin and streptomycin resistance) previously converted into a competent state by calcium chloride treatment was transformed independently with two plasmids BjRS-Sac(+)-121 and BjRS-Sac(-)-121 prepared in Example 8. The transformants were selected on LB medium containing 50 µg/ml rifampicin and 25 µg/ml kanamycin by utilizing the kanamycin resistant character conferred by the kanamycin resistant gene (neomycin phosphotransferase, NPTII) of the introduced plasmids.

The transformant Agrobacterium obtained (*Agrobacterium tumefaciens* strain LBA4404: rifampicin and streptomycin resistant) was cultured on LB medium containing 50 µg/ml rifampicin and 25 µg/ml kanamycin at 28°C for a whole day and night, and the culture was used for the transformation of mustard by the method described below.

The seeds of mustard were aseptically sowed on 1/2 MS medium containing 2% sucrose and 0.7% agar. After one week, cotyledons and petioles of sprouting plants were cut out with a scalpel, and transferred to MS medium containing 3% sucrose, 0.7% agar, 4.5 µM BA, 0.05 µM 2.4-D and 3.3 µM $AgNO_3$, followed by preculture for 1 day. The precultured cotyledons and petioles were transferred in a 1000-fold dilution of the Agrobacterium culture broth and allowed to stand for 5 minutes. The cotyledons and petioles were transferred again to the same

medium as used in the preculture, and cultured for 3 to 4 days. The cultured cotyledons and petioles were transferred to MS medium containing 3% sucrose, 4.5 μM BA, 0.05 μM 2.4-D, 3.3 μM $AgNO_3$ and 500 mg/l cefotaxim, and shaken for 1 day to remove microbial cells. The cotyledons and petioles thus treated were transferred to MS medium containing 3% sucrose, 0.7% agar, 4.5 μM BA, 0.05 μM 2.4-D, 3.3 μM $AgNO_3$, 100 mg/l cefotaxim and 20 mg/l kanamycin, and cultured for 3 to 4 weeks. The cotyledons and petioles were transferred to MS medium containing 3% sucrose, 0.7% agar, 4.5 μM BA, 0.05 μM 2.4-D, 100 mg/l cefotaxim and 20 mg/l kanamycin, and cultivated. The cultivation on this medium was continued with subculturing at intervals of 3 to 4 weeks. When shoots are began to regenerate, these shoots are subcultured on MS medium containing 3% sucrose, 0.7% agar and 20 mg/l kanamycin, and cultivated for 3 to 4 weeks. The rooting plants are transferred to vermiculite : peat moss = 1 : 1, and cultivated at 21°C to 22°C in a cycle of day/night = 12 hours : 12 hours. With the progress of plant body growth, the plants are grown with cultivation soil.

BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO: 1 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene of the present invention.

SEQ ID NO: 2 shows a nucleotide sequence of the raffinose synthase gene of the present invention.

SEQ ID NO: 3 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from beet.

SEQ ID NO: 4 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from beet.

SEQ ID NO: 5 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from mustard.

SEQ ID NO: 6 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from mustard.

SEQ ID NO: 7 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from rapeseed.

SEQ ID NO: 8 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from rapeseed.

List 1: (SEQ ID NOS: 9+10)

The nucleotide sequences shown in List 1 are examples of the typical primers used in the amplification of a DNA fragment having a raffinose synthase gene. All of these sequences are based on the nucleotide sequence of SEQ ID NO: 2. Primer 11 (SEQ ID NO: 9) is a sense primer and Primer 12 (SEQ ID NO: 10) is an antisense primer. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

List 2:

The nucleotide sequences shown in List 2 are examples (SEQ ID NOS: 11-14) of the typical primers used in the amplification of a cDNA of a raffinose synthase gene. Primer 21 (SEQ ID NO: 11) is a sense primer corresponding to the 5'-terminus of the beet-derived raffinose synthase gene. Primer 22 (SEQ ID NO: 12) is an antisense primer corresponding to the 3'-terminus. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

Primer 23 (SEQ ID NO: 13) is a sense primer corresponding to the N-terminus of the open reading frame, and primer 24 (SEQ ID NO: 14) is an antisense primer corresponding to the C-terminus.

List 3:

Among the nucleotide sequences shown in List 3, primers 31 and 32 are typical primers used in the amplification of a DNA having the partial nucleotide sequence of a raffinose synthase gene. Primer 31 (SEQ ID NO: 15) is a sense primer used in the amplification of a DNA having the partial nucleotide sequence of a raffinose synthase gene from mustard and rapeseed and primer 32 (SEQ ID NO: 16) is an antisense primer. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

Primers 33 and 34 (SEQ ID NO: 17) (SEQ ID NO: 18) are the typical primers used in the amplification of a cDNA of a raffinose synthase gene of mustard. Primers 33 and 34 (SEQ ID NO: 17) (SEQ ID NO: 18) are both based on the nucleotide sequence of

raffinose synthase gene in the non-translated region. Primer 33 (SEQ ID NO:17) is a sense primer corresponding to the 5'-terminal non-translated region of the mustard-derived raffinose synthase gene. Primer 34 (SEQ ID NO:18) is an antisense primer corresponding to the 3'-terminal non-translated region.

Primers 35 (SEQ ID NO:19) and 36 (SEQ ID NO:20) are typical primers used in the amplification of an open reading frame coding for the amino acid sequence of a raffinose synthase protein in the cDNA of a raffinose synthase gene. Primer 35 (SEQ ID NO:19) is a sense primer corresponding to the 5'-terminus of the above open reading frame. Primer 36 (SEQ ID NO:20) is an antisense primer corresponding to the 3'-terminus. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

List 4: (SEQ ID NOS:21-22)

The nucleotide sequences shown in List 4 are of the primers used in the cloning of a DNA fragment having the present raffinose synthase gene. The base represented by the symbol "i" is inosine. The bases shown in parentheses mean that a mixture of those bases is used in the synthesis. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 5: (SEQ ID NO:23)

The nucleotide sequence shown in List 5 is of the primer used in the analysis of a nucleotide sequence of the present

raffinose synthase gene. 5-SC-2 (SEQ ID NO:23) is used in the analysis of the present nucleotide sequence in the 3'-terminal region.

List 6

The nucleotide sequences shown in List 6 (SEQ ID NOS:24-27) are of the primers used in the analysis of the present raffinose synthase gene of beet. The base represented by the symbol "i" is inosine. The bases shown in parentheses mean that a mixture of those bases was used in the synthesis. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 7 (SEQ ID NOS:28 & 35)

The nucleotide sequences shown in List 7 are of the primers synthesized on the partial nucleotide sequences of the beet raffinose synthase gene. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 8: (SEQ ID NOS:36+37)

The nucleotide sequences shown in List 8 are of the primers used in the analysis of the cDNA nucleotide sequence of a raffinose synthase gene of mustard. The base represented by the symbol "i" is inosine. The bases shown in parentheses mean that a mixture of those bases. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 9:

(SEQ ID NOS: 38+39)

The nucleotide sequences shown in List 9 are of the primers synthesized on the partial nucleotide sequences of the mustard raffinose synthase gene. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 10: (SEQ ID NOS: 40-46)

The nucleotide sequences shown in List 10 are of the primers used in the analysis of the nucleotide sequences of raffinose synthase gene of mustard and rapeseed. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 11: (SEQ ID NOS 47+48)

The nucleotide sequences shown in List 11 are of the primers used in the amplification of 5'-terminal region of a mustard raffinose synthase gene. 11-SacI-BjN (SEQ ID NO: 47) is a primer whose SacI restriction site is added to the nucleotide sequence represented by the 4th to 29th nucleotides in SEQ ID NO: 6. 11-SacI-BjintRV (SEQ ID NO: 48) is an antisense primer having a nucleotide sequence corresponding to the nucleotide sequence represented by the 1164th to 1188th nucleotides in SEQ ID NO: 6.

List 12: (SEQ ID NOS: 49-50)

The nucleotide sequences shown in List 12 are of the adapters added to a mustard cDNA. These synthetic DNA take a double-stranded form when mixed together because they are complementary strands. This adapter has cohesive ends of cleavage

sites for the restriction enzymes BamHI and SacI on both termini, and contains the restriction sites for the restriction enzymes BamHI and SacI in the double-stranded region.

List 13: (SEQ ID NOS: 51-53)

The nucleotide sequences shown in List 13 are of the primers used in the confirmation of inserting direction of the mustard-derived raffinose synthase gene. 13-35S-3 (SEQ ID NO: 51) is a primer of sense to 35S promoter. 13-B-2RV (SEQ ID NO: 52) is an antisense primer having the nucleotide sequence represented by the 593rd to 622nd nucleotides of SEQ ID NO: 6, 13-B-8 (SEQ ID NO: 53) is a sense primer having the nucleotide sequence represented by the 1110th to 1138th nucleotides in SEQ ID NO: 6.

As described hereinabove, according to the present invention, it is possible to provide raffinose synthase genes which can be utilized in techniques for varying expression level and activity of raffinose synthase in plants.

SEQUENCE LISTING FREE TEXT

SEQ ID NO: 9 to SEQ ID NO: 20: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 21 and SEQ ID NO: 22: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

SEQ ID NO: 23: Designated oligonucleotide primer to

obtain raffinose synthase gene.

SEQ ID NO: 24 to SEQ ID NO: 27: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, y is t or c, r is a or g.

SEQ ID NO: 28 to SEQ ID NO: 35: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 36 and SEQ ID NO: 37: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

SEQ ID NO: 38 to SEQ ID NO: 48: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 49 and SEQ ID NO: 50: Designed oligonucleotide linker to obtain raffinose synthase gene.

SEQ ID NO: 51 to SEQ ID NO: 53: Designed oligonucleotide primer to confirm direction of the inserted raffinose synthase gene.

SEQUENCE LISTING

<110> Eijiro WATANABE et al.; Sumitomo Chemical Company Limited

<120> Raffinose Synthase Genes and Their Use

<150> JP 10/120550
<151> 1998-04-30

<150> JP 10/120551
<151> 1998-04-30

<150> JP 10/345590
<151> 1998-12-04

<150> JP 10/351246
<151> 1998-12-10

<160> 53

<210> 1
<211> 265
<212> PRT
<213> Glycine max

<400> 1

```
Gln Ser Asp His Ala Cys Ala Glu Phe His Ala Ala Ser Arg Ala Ile
                  5                  10                  15
Ser Gly Gly Pro Ile Tyr Val Ser Asp Ser Val Gly Lys His Asn Phe
             20                  25                  30
Lys Leu Leu Lys Lys Leu Val Leu Pro Asp Gly Ser Ile Leu Arg Cys
         35                  40                  45
Gln His Tyr Ala Leu Pro Thr Arg Asp Cys Leu Phe Val Asp Pro Leu
     50                  55                  60
His Asp Gly Lys Thr Met Leu Lys Ile Trp Asn Leu Asn Lys Cys Ser
 65                  70                  75                  80
Gly Val Leu Gly Leu Phe Asn Cys Gln Gly Gly Gly Trp Cys Pro Val
```

85 90 95
Thr Arg Arg Asn Lys Ser Ser Ser Asp Tyr Ser His Ser Val Thr Cys
100 105 110
Phe Ala Ser Pro Gln Asp Ile Glu Trp Gly Lys Gly Lys His Pro Val
115 120 125
Cys Ile Lys Gly Val Asp Val Phe Ala Val Tyr Met Phe Lys Asp Asp
130 135 140
Lys Leu Lys Leu Leu Lys Tyr Thr Glu Ser Val Glu Val Ser Leu Glu
145 150 155 160
Pro Phe Ser Cys Glu Leu Leu Thr Val Ser Pro Val Val Ile Leu Pro
165 170 175
Arg Lys Ser Ile Gln Phe Ala Pro Ile Gly Leu Val Asn Met Leu Asn
180 185 190
Ser Gly Gly Ser Ile Met Ser Leu Glu Phe Asp Gln Gln Glu Asn Leu
195 200 205
Ala Arg Ile Gly Val Arg Gly His Gly Glu Met Arg Val Phe Ala Ser
210 215 220
Glu Lys Pro Glu Ser Val Lys Ile Asp Gly Glu Ser Val Glu Phe Asp
225 230 235 240
Tyr Val Asp Arg Thr Val Arg Leu Gln Val Ser Trp Pro Cys Ser Ser
245 250 255
Arg Leu Ser Val Val Glu Tyr Leu Phe
260 265

<210> 2
<211> 928
<212> DNA
<213> Glycine max

<220>
<221> CDS
<222> (2)...(799)

<400> 2
c caa tct gat cat gct tgt gcc gaa ttc cac gct gct tct aga gcc 46
Gln Ser Asp His Ala Cys Ala Glu Phe His Ala Ala Ser Arg Ala
5 10 15
att tct ggt gga cca att tat gta agc gac tct gtt gga aaa cac aac 94
Ile Ser Gly Gly Pro Ile Tyr Val Ser Asp Ser Val Gly Lys His Asn

```
                                20                  25                  30
    ttc aag ttg ctt aag aag ctt gtt cta cct gat ggc tcc att ttg cgg    142
    Phe Lys Leu Leu Lys Lys Leu Val Leu Pro Asp Gly Ser Ile Leu Arg
                            35                  40                  45
5   tgt caa cat tat gca ctt ccc acc cga gac tgc tta ttt gta gat cct    190
    Cys Gln His Tyr Ala Leu Pro Thr Arg Asp Cys Leu Phe Val Asp Pro
                        50                  55                  60
    tta cat gat ggg aaa aca atg ctc aaa att tgg aac ctc aat aaa tgt    238
    Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp Asn Leu Asn Lys Cys
10              65                  70                  75
    tcc ggg gtt ttg ggt ctg ttc aat tgc caa gga gga ggt tgg tgc cct    286
    Ser Gly Val Leu Gly Leu Phe Asn Cys Gln Gly Gly Gly Trp Cys Pro
                80                  85                  90                  95
    gtt act agg cga aac aag agt agc tct gac tat tca cac tcc gtg act    334
15  Val Thr Arg Arg Asn Lys Ser Ser Ser Asp Tyr Ser His Ser Val Thr
                            100                 105                 110
    tgc ttt gca agt cct caa gac att gaa tgg ggc aaa ggg aag cac cca    382
    Cys Phe Ala Ser Pro Gln Asp Ile Glu Trp Gly Lys Gly Lys His Pro
                        115                 120                 125
20  gtt tgc atc aaa ggg gtg gac gta ttt gct gtg tac atg ttt aag gac    430
    Val Cys Ile Lys Gly Val Asp Val Phe Ala Val Tyr Met Phe Lys Asp
                    130                 135                 140
    gac aag ttg aag ctg ctg aag tac aca gag agt gta gaa gtt tct ctt    478
    Asp Lys Leu Lys Leu Leu Lys Tyr Thr Glu Ser Val Glu Val Ser Leu
25              145                 150                 155
    gag cct ttt agt tgt gag ctt ttg acc gtt tct cca gtg gtg atc tta    526
    Glu Pro Phe Ser Cys Glu Leu Leu Thr Val Ser Pro Val Val Ile Leu
    160                 165                 170                 175
    ccc aga aaa tca atc caa ttt gcc cca att gga ttg gta aac atg ctc    574
30  Pro Arg Lys Ser Ile Gln Phe Ala Pro Ile Gly Leu Val Asn Met Leu
                        180                 185                 190
    aac tct ggg ggc tct att atg tca ttg gaa ttt gat caa cag gaa aat    622
    Asn Ser Gly Gly Ser Ile Met Ser Leu Glu Phe Asp Gln Gln Glu Asn
                    195                 200                 205
35  ttg gcg agg att ggg gtg aga gga cat ggg gaa atg agg gta ttt gca    670
    Leu Ala Arg Ile Gly Val Arg Gly His Gly Glu Met Arg Val Phe Ala
                210                 215                 220
    tca gag aag cca gag agt gtc aag att gat gga gaa tct gtg gaa ttt    718
    Ser Glu Lys Pro Glu Ser Val Lys Ile Asp Gly Glu Ser Val Glu Phe
```

```
    225                 230                 235
gat tat gtt gat aga acc gtg agg ctc caa gtc tcg tgg cct tgt tct    766
Asp Tyr Val Asp Arg Thr Val Arg Leu Gln Val Ser Trp Pro Cys Ser
240                 245                 250                 255
tcg agg ttg tcc gta gtc gag tat ttg ttc tga atcatgattt ggtgtccgag  819
Ser Arg Leu Ser Val Val Glu Tyr Leu Phe
                260                 265
agagccgtgt aatgttcaca taaactgact taagtgcatt aagcaaatcc accttaaata  879
atagtgcata actttgttcc aaaaaaaaaa aaaaaaaaaa aaaaaaaaa              928
```

<210> 3
<211> 783
<212> PRT
<213> Beta vulgaris L.

<400> 3

```
Met Ala Pro Ser Phe Ser Lys Glu Asn Ser Lys Thr Cys Asp Glu Val
                5                  10                  15
Ala Asn His Asp Asp Cys Asn Thr Cys Pro Ile Ile Ser Leu Glu Glu
            20                  25                  30
Ser Asn Phe Met Val Asn Gly His Val Ile Leu Ser Gln Val Pro Ser
        35                  40                  45
Asn Ile Thr Ala Ile Ser Lys Met Gly Phe Asp Gly Leu Phe Val Gly
    50                  55                  60
Phe Asp Ala Pro Glu Pro Lys Ala Arg His Val Val Ser Val Gly Gln
65                  70                  75                  80
Leu Lys Gly Ile Pro Phe Met Ser Ile Phe Arg Phe Lys Val Trp Trp
                85                  90                  95
Thr Thr His Trp Thr Gly Ser Asn Gly Arg Asp Leu Glu His Glu Thr
            100                 105                 110
Gln Ile Leu Ile Leu Asp Lys Ser Asp Glu Gly Leu Gly Arg Pro Tyr
        115                 120                 125
Ile Val Ile Leu Pro Leu Ile Glu Gly Pro Phe Arg Ala Ser Leu Gln
    130                 135                 140
Pro Gly Ser Val Asp Asp Tyr Val Asp Ile Cys Val Glu Ser Gly Ser
145                 150                 155                 160
Thr Lys Val Val Gly Asp Ser Phe Arg Ala Val Leu Tyr Ile Arg Ala
                165                 170                 175
Gly Pro Asp Pro Phe Lys Leu Ile Lys Asp Thr Met Lys Glu Val Gln
```

```
                180                 185                 190
Ala His Leu Gly Thr Phe Lys Leu Leu Asp Asp Lys Thr Pro Pro Gly
        195                 200                 205
Ile Val Asp Lys Phe Gly Trp Cys Thr Trp Asp Ala Phe Tyr Leu Lys
    210                 215                 220
Val Glu Pro Tyr Gly Val Trp Glu Gly Val Lys Gly Leu Val Glu Asn
225                 230                 235                 240
Gly Val Pro Pro Gly Leu Val Leu Ile Asp Asp Gly Trp Gln Ser Ile
                245                 250                 255
Cys His Asp Asp Asp Pro Ile Thr Asp Gln Glu Gly Ile Asn Arg Thr
            260                 265                 270
Ser Ala Gly Glu Gln Met Pro Cys Arg Leu Ile Lys Tyr Glu Glu Asn
        275                 280                 285
Phe Lys Phe Arg Asp Tyr Lys Ser Pro Asn Ile Met Gly His Glu Asp
    290                 295                 300
His Pro Asn Met Gly Met Arg Ala Phe Val Arg Asp Leu Lys Glu Glu
305                 310                 315                 320
Phe Lys Thr Val Glu His Val Tyr Val Trp His Ala Phe Thr Gly Tyr
                325                 330                 335
Trp Gly Gly Val Arg Pro Asn Val Pro Gly Leu Pro Glu Ala Gln Val
            340                 345                 350
Val Thr Pro Lys Leu Ser Pro Gly Leu Glu Met Thr Met Glu Asp Leu
        355                 360                 365
Ala Val Asp Lys Ile Val Asn Asn Gly Ile Gly Leu Val Gln Pro Asp
    370                 375                 380
Lys Ala Gln Glu Leu Tyr Glu Gly Leu His Ser His Leu Glu Asn Cys
385                 390                 395                 400
Gly Ile Asp Gly Val Lys Val Asp Val Ile His Leu Leu Glu Met Met
                405                 410                 415
Ala Glu Asp Tyr Gly Gly Arg Val Glu Leu Ala Lys Thr Tyr Tyr Lys
            420                 425                 430
Ala Ile Thr Glu Ser Val Arg Lys His Phe Lys Gly Asn Gly Val Ile
        435                 440                 445
Ala Ser Met Glu Gln Cys Asn Asp Phe Met Leu Leu Gly Thr Glu Thr
    450                 455                 460
Ile Cys Leu Gly Arg Val Gly Asp Asp Phe Trp Pro Thr Asp Pro Ser
465                 470                 475                 480
Gly Asp Ile Asn Gly Thr Tyr Trp Leu Gln Gly Cys His Met Val His
                485                 490                 495
```

Cys Ala Tyr Asn Ser Leu Trp Met Gly Asn Phe Ile His Pro Asp Trp
500 505 510
Asp Met Phe Gln Ser Thr His Pro Cys Ala Glu Phe His Ala Ala Ser
515 520 525
Arg Ala Ile Ser Gly Gly Pro Ile Tyr Val Ser Asp Val Val Gly Lys
530 535 540
His Asn Ile Pro Leu Leu Lys Arg Leu Val Leu Ala Asp Gly Ser Ile
545 550 555 560
Leu Arg Cys Glu Tyr His Ala Leu Pro Thr Lys Asp Cys Leu Phe Val
565 570 575
Asp Pro Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp Asn Leu Asn
580 585 590
Lys Tyr Asn Gly Val Leu Gly Val Phe Asn Cys Gln Gly Gly Gly Trp
595 600 605
Ser Arg Glu Ser Arg Lys Asn Leu Cys Phe Ser Glu Tyr Ser Lys Pro
610 615 620
Ile Ser Cys Lys Thr Ser Pro Lys Asp Val Glu Trp Glu Asn Gly His
625 630 635 640
Lys Pro Phe Pro Ile Lys Gly Val Glu Cys Phe Ala Met Tyr Phe Thr
645 650 655
Lys Glu Lys Lys Leu Ile Leu Ser Gln Leu Ser Asp Thr Ile Glu Ile
660 665 670
Ser Leu Asp Pro Phe Asp Tyr Glu Leu Ile Val Val Ser Pro Met Thr
675 680 685
Ile Leu Pro Trp Glu Ser Ile Ala Phe Ala Pro Ile Gly Leu Val Asn
690 695 700
Met Leu Asn Ala Gly Gly Ala Val Lys Ser Leu Asp Ile Ser Glu Asp
705 710 715 720
Asn Glu Asp Lys Met Val Gln Val Gly Ile Lys Gly Ala Gly Glu Met
725 730 735
Met Val Tyr Ser Ser Glu Lys Pro Lys Ala Cys Arg Val Asn Gly Glu
740 745 750
Asp Met Glu Phe Glu Tyr Glu Glu Ser Met Ile Lys Val Gln Val Thr
755 760 765
Trp Asn His Asn Ser Gly Gly Phe Thr Thr Val Glu Tyr Leu Phe
770 775 780 783

<210> 4
<211> 2690

<212> DNA
<213> Beta vulgaris L.

<220>
<221> CDS
<222> (236)...(2587)

<400> 4

```
ctaccaaatt ccacaactta aagttcacct caatctttat tccatttttc ctccctaaac      60
ttcattgtta agattttgta attgaattca aattcttaat tctgaatttt gtcatttttt     120
ttgtggggat atttataact atcatattat ttgtgtagat cattctacaa aaaagagagt     180
gagtttttt agctcttatt tcctaagaaa ttaatagcaa aagttttgca taact atg        238
                                                              Met
gct cca agc ttt agc aag gaa aat tcc aag acg tgt gat gag gtt gca        286
Ala Pro Ser Phe Ser Lys Glu Asn Ser Lys Thr Cys Asp Glu Val Ala
                5                  10                  15
aac cat gat gat tgc aac acg tgt cca ata att tcc ttg gaa gaa tca        334
Asn His Asp Asp Cys Asn Thr Cys Pro Ile Ile Ser Leu Glu Glu Ser
            20                  25                  30
aac ttc atg gtg aat ggt cac gtg ata ttg tcc caa gtt cca tcc aac        382
Asn Phe Met Val Asn Gly His Val Ile Leu Ser Gln Val Pro Ser Asn
        35                  40                  45
atc acg gcc att agt aaa atg ggt ttt gat ggg ctt ttt gtg ggt ttt        430
Ile Thr Ala Ile Ser Lys Met Gly Phe Asp Gly Leu Phe Val Gly Phe
    50                  55                  60                  65
gat gct cca gag ccc aag gcc cgg cac gtt gta tcc gtg ggc cag ctc        478
Asp Ala Pro Glu Pro Lys Ala Arg His Val Val Ser Val Gly Gln Leu
                70                  75                  80
aag gga att ccc ttc atg agt atc ttc agg ttc aag gta tgg tgg act        526
Lys Gly Ile Pro Phe Met Ser Ile Phe Arg Phe Lys Val Trp Trp Thr
            85                  90                  95
acc cat tgg act ggg tcc aat ggg cgg gac ctt gag cat gag acc caa        574
Thr His Trp Thr Gly Ser Asn Gly Arg Asp Leu Glu His Glu Thr Gln
        100                 105                 110
att ctc atc ctt gat aag tca gat gaa ggt ttg ggc cgt ccc tat att        622
Ile Leu Ile Leu Asp Lys Ser Asp Glu Gly Leu Gly Arg Pro Tyr Ile
    115                 120                 125
gtg atc ctc cca ttg atc gaa ggc cca ttt cgg gca tct ctc cag ccg        670
Val Ile Leu Pro Leu Ile Glu Gly Pro Phe Arg Ala Ser Leu Gln Pro
```

```
    130                 135                 140                 145
    ggt tct gtt gat gac tat gtg gat ata tgt gtt gag agt ggg tcc act      718
    Gly Ser Val Asp Asp Tyr Val Asp Ile Cys Val Glu Ser Gly Ser Thr
                    150                 155                 160
    aaa gtt gtc gga gac tcg ttc cgg gct gtt ctt tat ata cgg gct ggg      766
    Lys Val Val Gly Asp Ser Phe Arg Ala Val Leu Tyr Ile Arg Ala Gly
                165                 170                 175
    cct gac cca ttt aag tta att aaa gat aca atg aag gaa gtc caa gcc      814
    Pro Asp Pro Phe Lys Leu Ile Lys Asp Thr Met Lys Glu Val Gln Ala
            180                 185                 190
    cat tta ggg act ttc aaa ctc tta gat gac aaa act cct cca gga ata      862
    His Leu Gly Thr Phe Lys Leu Leu Asp Asp Lys Thr Pro Pro Gly Ile
        195                 200                 205
    gtg gac aag ttt gga tgg tgt aca tgg gat gca ttt tac ctc aaa gta      910
    Val Asp Lys Phe Gly Trp Cys Thr Trp Asp Ala Phe Tyr Leu Lys Val
    210                 215                 220                 225
    gag ccw tat ggt gtt tgg gaa gga gtt aaa gga ctc gtc gaa aac ggg      958
    Glu Pro Tyr Gly Val Trp Glu Gly Val Lys Gly Leu Val Glu Asn Gly
                    230                 235                 240
    gtc cca ccc ggt ctc gta ctc att gat gat ggg tgg caa tct att tgt      1006
    Val Pro Pro Gly Leu Val Leu Ile Asp Asp Gly Trp Gln Ser Ile Cys
                245                 250                 255
    cat gac gat gat ccg att acc gac caa gaa ggg ata aac cgg act tct      1054
    His Asp Asp Asp Pro Ile Thr Asp Gln Glu Gly Ile Asn Arg Thr Ser
            260                 265                 270
    gcc ggc gag caa atg cca tgt aga ttg atc aag tac gag gaa aac ttc      1102
    Ala Gly Glu Gln Met Pro Cys Arg Leu Ile Lys Tyr Glu Glu Asn Phe
        275                 280                 285
    aag ttt agg gac tat aaa agc cca aat att atg ggc cat gag gat cat      1150
    Lys Phe Arg Asp Tyr Lys Ser Pro Asn Ile Met Gly His Glu Asp His
    290                 295                 300                 305
    ccc aat atg gga atg agg gcc ttt gtt agg gac ctt aag gag gag ttc      1198
    Pro Asn Met Gly Met Arg Ala Phe Val Arg Asp Leu Lys Glu Glu Phe
                    310                 315                 320
    aaa act gtt gag cat gtg tat gtt tgg cat gct ttt acg ggc tat tgg      1246
    Lys Thr Val Glu His Val Tyr Val Trp His Ala Phe Thr Gly Tyr Trp
                325                 330                 335
    gga ggg gta agg ccc aat gtt cca ggc cta ccr gag gcc caa gta gta      1294
    Gly Gly Val Arg Pro Asn Val Pro Gly Leu Pro Glu Ala Gln Val Val
```

```
          340                 345                 350
acc cca aag ctt tcc ccg ggt ctt gag atg aca atg gaa gat cta gct   1342
Thr Pro Lys Leu Ser Pro Gly Leu Glu Met Thr Met Glu Asp Leu Ala
    355                 360                 365
gtg gat aaa att gtt aat aat ggt att ggg ctt gtc cag cct gat aag   1390
Val Asp Lys Ile Val Asn Asn Gly Ile Gly Leu Val Gln Pro Asp Lys
370                 375                 380                 385
gcc caa gaa ctt tat gaa ggg ttg cat tct cat ttg gaa aat tgt ggg   1438
Ala Gln Glu Leu Tyr Glu Gly Leu His Ser His Leu Glu Asn Cys Gly
                390                 395                 400
att gat gga gtc aaa gtt gat gtc atc cat ttg ttg gag atg atg gca   1486
Ile Asp Gly Val Lys Val Asp Val Ile His Leu Leu Glu Met Met Ala
            405                 410                 415
gag gac tat gga gga aga gtt gaa cta gca aaa aca tac tat aag gca   1534
Glu Asp Tyr Gly Gly Arg Val Glu Leu Ala Lys Thr Tyr Tyr Lys Ala
        420                 425                 430
ata aca gaa tca gtg cgt aag cat ttc aaa ggc aac ggt gtg att gct   1582
Ile Thr Glu Ser Val Arg Lys His Phe Lys Gly Asn Gly Val Ile Ala
    435                 440                 445
agc atg gag cag tgc aac gat ttc atg ctc ctt ggt act gag acc att   1630
Ser Met Glu Gln Cys Asn Asp Phe Met Leu Leu Gly Thr Glu Thr Ile
450                 455                 460                 465
tgt ctt ggt cgc gtt ggg gat gac ttt tgg cca act gat ccg tct gga   1678
Cys Leu Gly Arg Val Gly Asp Asp Phe Trp Pro Thr Asp Pro Ser Gly
                470                 475                 480
gat ata aat ggt aca tat tgg ctc caa ggc tgt cat atg gtg cat tgt   1726
Asp Ile Asn Gly Thr Tyr Trp Leu Gln Gly Cys His Met Val His Cys
            485                 490                 495
gcc tac aat agc tta tgg atg gga aac ttt ata cac cct gac tgg gac   1774
Ala Tyr Asn Ser Leu Trp Met Gly Asn Phe Ile His Pro Asp Trp Asp
        500                 505                 510
atg ttc caa tct aca cac cct tgt gct gaa ttt cat gct gca tct cgt   1822
Met Phe Gln Ser Thr His Pro Cys Ala Glu Phe His Ala Ala Ser Arg
    515                 520                 525
gcg att tct ggt gga cca att tat gtt agt gat gtt gtt ggc aag cat   1870
Ala Ile Ser Gly Gly Pro Ile Tyr Val Ser Asp Val Val Gly Lys His
530                 535                 540                 545
aac atc ccc ttg ctc aaa agg ctc gtc ttg gct gat ggt tcg atc ctt   1918
Asn Ile Pro Leu Leu Lys Arg Leu Val Leu Ala Asp Gly Ser Ile Leu
```

```
                                550                 555                 560
     cgt tgc gag tac cat gca ctt cct act aag gat tgc cta ttt gta gat      1966
     Arg Cys Glu Tyr His Ala Leu Pro Thr Lys Asp Cys Leu Phe Val Asp
                 565                 570                 575
     cct ttg cac gat ggc aaa aca atg ctc aaa att tgg aac ctc aac aag      2014
     Pro Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp Asn Leu Asn Lys
             580                 585                 590
     tac aat gga gtg ctt gga gtc ttc aat tgc caa gga gga ggg tgg agc      2062
     Tyr Asn Gly Val Leu Gly Val Phe Asn Cys Gln Gly Gly Gly Trp Ser
         595                 600                 605
     cgt gag tct cga aaa aat cta tgt ttc tca gag tat tca aaa cct att      2110
     Arg Glu Ser Arg Lys Asn Leu Cys Phe Ser Glu Tyr Ser Lys Pro Ile
     610                 615                 620                 625
     tcc tgc aag aca agt cca aaa gat gtt gaa tgg gag aac gga cac aag      2158
     Ser Cys Lys Thr Ser Pro Lys Asp Val Glu Trp Glu Asn Gly His Lys
                     630                 635                 640
     cca ttc ccc atc aaa gga gtg gaa tgt ttt gcc atg tac ttc acc aag      2206
     Pro Phe Pro Ile Lys Gly Val Glu Cys Phe Ala Met Tyr Phe Thr Lys
                 645                 650                 655
     gaa aaa aag cta atc ctc tca caa cta tct gac acc att gaa ata tca      2254
     Glu Lys Lys Leu Ile Leu Ser Gln Leu Ser Asp Thr Ile Glu Ile Ser
             660                 665                 670
     ctt gat ccc ttc gat tac gag ctt att gta gtc tct ccg atg aca att      2302
     Leu Asp Pro Phe Asp Tyr Glu Leu Ile Val Val Ser Pro Met Thr Ile
         675                 680                 685
     cta ccc tgg gag tcg atc gca ttt gca ccc ata gga tta gta aac atg      2350
     Leu Pro Trp Glu Ser Ile Ala Phe Ala Pro Ile Gly Leu Val Asn Met
     690                 695                 700                 705
     ctc aac gcc gga ggg gca gtc aag tct ttg gac atc agt gag gat aat      2398
     Leu Asn Ala Gly Gly Ala Val Lys Ser Leu Asp Ile Ser Glu Asp Asn
                     710                 715                 720
     gag gat aag atg gtt cag gtt ggt att aaa ggg gcc gga gaa atg atg      2446
     Glu Asp Lys Met Val Gln Val Gly Ile Lys Gly Ala Gly Glu Met Met
                 725                 730                 735
     gtt tat tca tca gaa aag cca aaa gcg tgt aga gtt aat gga gaa gac      2494
     Val Tyr Ser Ser Glu Lys Pro Lys Ala Cys Arg Val Asn Gly Glu Asp
             740                 745                 750
     atg gag ttt gag tat gaa gag agc atg att aag gtt caa gtt aca tgg      2542
     Met Glu Phe Glu Tyr Glu Glu Ser Met Ile Lys Val Gln Val Thr Trp
```

```
    755                 760                 765
aac cat aac tca ggt ggt ttt acc act gtt gag tac tta ttt tga gcttg   2592
Asn His Asn Ser Gly Gly Phe Thr Thr Val Glu Tyr Leu Phe
770                 775                 780
aagctaatct aagtctttac ttaatgagtg atgtaactga gtagttgact tgagagtaca   2652
gtatgtgtga agcttattat tccaaaaaaa aaaaaaaa                            2690

<210> 5
<211> 777
<212> PRT
<213> Brassica juncea

<400> 5
Met Ala Pro Pro Ser Val Ile Lys Ser Asp Ala Ala Val Asn Gly Ile
 1               5                  10                  15
Asp Leu Ser Gly Lys Pro Leu Phe Arg Leu Glu Gly Ser Asp Leu Leu
            20                  25                  30
Ala Asn Gly His Val Val Leu Thr Asp Val Pro Val Asn Val Thr Val
        35                  40                  45
Thr Ala Ser Pro Tyr Leu Ala Asp Lys Asp Gly Glu Pro Val Asp Ala
    50                  55                  60
Ser Ala Gly Ser Phe Ile Gly Phe Asn Leu Asp Gly Glu Pro Arg Ser
65                  70                  75                  80
Arg His Val Ala Ser Ile Gly Lys Leu Arg Asp Ile Arg Phe Met Ser
                85                  90                  95
Ile Phe Arg Phe Lys Val Trp Trp Thr Thr His Trp Val Gly Ser Lys
            100                 105                 110
Gly Ser Asp Ile Glu Asn Glu Thr Gln Ile Ile Ile Leu Glu Asn Ser
        115                 120                 125
Gly Ser Gly Arg Pro Tyr Val Leu Leu Leu Pro Leu Leu Glu Gly Ser
    130                 135                 140
Phe Arg Ser Ser Phe Gln Pro Gly Glu Asp Asp Asp Val Ala Val Cys
145                 150                 155                 160
Val Glu Ser Gly Ser Thr Gln Val Thr Gly Ser Glu Phe Arg Gln Val
                165                 170                 175
Val Tyr Val His Ala Gly Asp Asp Pro Phe Lys Leu Val Lys Asp Ala
            180                 185                 190
Met Lys Val Val Arg Val His Met Asn Thr Phe Lys Leu Leu Glu Glu
        195                 200                 205
```

```
Lys Thr Pro Pro Gly Ile Val Asp Lys Phe Gly Trp Cys Thr Trp Asp
    210                 215                 220
Ala Phe Tyr Leu Thr Val Asn Pro Asp Gly Val His Lys Gly Val Lys
225                 230                 235                 240
Cys Leu Val Asp Gly Gly Cys Pro Pro Gly Leu Val Leu Ile Asp Asp
                245                 250                 255
Gly Trp Gln Ser Ile Gly His Asp Ser Asp Gly Ile Asp Val Glu Gly
            260                 265                 270
Met Ser Cys Thr Val Ala Gly Glu Gln Met Pro Cys Arg Leu Leu Lys
        275                 280                 285
Phe Gln Glu Asn Phe Lys Phe Arg Asp Tyr Val Ser Pro Lys Asp Lys
    290                 295                 300
Asn Glu Val Gly Met Lys Ala Phe Val Arg Asp Leu Lys Glu Glu Phe
305                 310                 315                 320
Ser Thr Val Asp Tyr Ile Tyr Val Trp His Ala Leu Cys Gly Tyr Trp
                325                 330                 335
Gly Gly Leu Arg Pro Gly Ala Pro Thr Leu Pro Pro Ser Thr Ile Val
            340                 345                 350
Arg Pro Glu Leu Ser Pro Gly Leu Lys Leu Thr Met Gln Asp Leu Ala
        355                 360                 365
Val Asp Lys Ile Val Asp Thr Gly Ile Gly Phe Val Ser Pro Asp Met
    370                 375                 380
Ala Asn Glu Phe Tyr Glu Gly Leu His Ser His Leu Gln Asn Val Gly
385                 390                 395                 400
Ile Asp Gly Val Lys Val Asp Val Ile His Ile Leu Glu Met Leu Cys
                405                 410                 415
Glu Lys Tyr Gly Gly Arg Val Asp Leu Ala Lys Ala Tyr Phe Lys Ala
            420                 425                 430
Leu Thr Ser Ser Val Asn Lys His Phe Asp Gly Asn Gly Val Ile Ala
        435                 440                 445
Ser Met Glu His Cys Asn Asp Phe Met Phe Leu Gly Thr Glu Ala Ile
    450                 455                 460
Ser Leu Gly Arg Val Gly Asp Asp Phe Trp Cys Thr Asp Pro Ser Gly
465                 470                 475                 480
Asp Ile Asn Gly Thr Tyr Trp Leu Gln Gly Cys His Met Val His Cys
                485                 490                 495
Ala Tyr Asn Ser Leu Trp Met Gly Asn Phe Ile Gln Pro Asp Trp Asp
            500                 505                 510
Met Phe Gln Ser Thr His Pro Cys Ala Glu Phe His Ala Ala Ser Arg
```

```
                515                 520                 525
Ala Ile Ser Gly Gly Pro Ile Tyr Ile Ser Asp Cys Val Gly Gln His
    530                 535                 540
Asp Phe Asp Leu Leu Lys Arg Leu Val Leu Pro Asp Gly Ser Ile Leu
545                 550                 555                 560
Arg Cys Glu His Tyr Ala Leu Pro Thr Arg Asp Arg Leu Phe Glu Asp
                565                 570                 575
Pro Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp Asn Leu Asn Lys
            580                 585                 590
Tyr Thr Gly Ile Ile Gly Ala Phe Asn Cys Gln Gly Gly Gly Trp Cys
        595                 600                 605
Arg Glu Thr Arg Arg Asn Gln Cys Phe Ser Gln Cys Val Asn Thr Leu
    610                 615                 620
Thr Ala Thr Thr Asn Pro Lys Asp Val Glu Trp Asn Ser Gly Asn Asn
625                 630                 635                 640
Pro Ile Ser Val Glu Asn Val Glu Glu Phe Ala Leu Phe Leu Ser Gln
                645                 650                 655
Ser Lys Lys Leu Val Leu Ser Gly Pro Asn Asp Asp Leu Glu Ile Thr
            660                 665                 670
Leu Glu Pro Phe Lys Phe Glu Leu Ile Thr Val Ser Pro Val Val Thr
        675                 680                 685
Ile Glu Gly Ser Ser Val Gln Phe Ala Pro Ile Gly Leu Val Asn Met
    690                 695                 700
Leu Asn Thr Ser Gly Ala Ile Arg Ser Leu Val Tyr His Glu Glu Ser
705                 710                 715                 720
Val Glu Ile Gly Val Arg Gly Ala Gly Glu Phe Arg Val Tyr Ala Ser
                725                 730                 735
Arg Lys Pro Ala Ser Cys Lys Ile Asp Gly Glu Val Val Glu Phe Gly
            740                 745                 750
Tyr Glu Glu Ser Met Val Met Val Gln Val Pro Trp Ser Ala Pro Glu
        755                 760                 765
Gly Leu Ser Ser Ile Lys Tyr Glu Phe
    770                 775     777
```

<210> 6
<211> 2690
<212> DNA
<213> Brassica juncea

<220>
<221> CDS
<222> (134)... (2467)

<400> 6

```
accaatccaa aatctcatca aataatcgca attaggggaa gtttacaaga ttcatcatct     60
ccgttactat ataactacgc tcttcttcct tcgcctaatc caacttaacc taaaaaccac    120
tctatcagcg aaa atg gct cca ccg agc gta att aaa tcc gat gct gca        169
                Met Ala Pro Pro Ser Val Ile Lys Ser Asp Ala Ala
                                  5                  10
gtc aac ggc att gac ctc tcc gga aag ccg ctt ttc cgg cta gag ggt      217
Val Asn Gly Ile Asp Leu Ser Gly Lys Pro Leu Phe Arg Leu Glu Gly
            15                  20                  25
tcc gat ctc cta gcc aat ggt cac gtt gtc tta acc gat gta ccg gtt      265
Ser Asp Leu Leu Ala Asn Gly His Val Val Leu Thr Asp Val Pro Val
        30                  35                  40
aac gtg act gtc act gct tca cct tac cta gct gac aaa gac gga gaa      313
Asn Val Thr Val Thr Ala Ser Pro Tyr Leu Ala Asp Lys Asp Gly Glu
    45                  50                  55                  60
ccg gtt gac gcc tcc gct ggt tca ttc atc ggg ttt aat ctc gac ggt      361
Pro Val Asp Ala Ser Ala Gly Ser Phe Ile Gly Phe Asn Leu Asp Gly
                65                  70                  75
gag cca cga agc cgc cac gtg gcg tcc atc ggt aaa ctc agg gat att      409
Glu Pro Arg Ser Arg His Val Ala Ser Ile Gly Lys Leu Arg Asp Ile
            80                  85                  90
cga ttc atg agc ata ttc cgt ttc aag gtt tgg tgg act act cac tgg      457
Arg Phe Met Ser Ile Phe Arg Phe Lys Val Trp Trp Thr Thr His Trp
        95                  100                 105
gtc ggt tcc aaa gga tcc gac atc gag aac gag acc cag atc atc atc      505
Val Gly Ser Lys Gly Ser Asp Ile Glu Asn Glu Thr Gln Ile Ile Ile
    110                 115                 120
ctc gag aac tcc ggg tcg ggt cgt cct tat gtt ctt ctt ctg ccg ctt      553
Leu Glu Asn Ser Gly Ser Gly Arg Pro Tyr Val Leu Leu Leu Pro Leu
125                 130                 135                 140
ctt gaa ggc tct ttc cgt tca tcc ttt cag cct ggg gaa gac gat gac      601
Leu Glu Gly Ser Phe Arg Ser Ser Phe Gln Pro Gly Glu Asp Asp Asp
                145                 150                 155
gtg gcg gtt tgt gtc gaa tcc ggg tcg acc cag gtg acc ggg tcg gag      649
Val Ala Val Cys Val Glu Ser Gly Ser Thr Gln Val Thr Gly Ser Glu
```

```
                160                 165                 170
ttt cgt caa gtt gtg tat gtt cac gcc gga gac gat ccg ttc aag ctc     697
Phe Arg Gln Val Val Tyr Val His Ala Gly Asp Asp Pro Phe Lys Leu
        175                 180                 185
gtg aaa gac gcg atg aag gtg gtt agg gtt cat atg aac acc ttc aag     745
Val Lys Asp Ala Met Lys Val Val Arg Val His Met Asn Thr Phe Lys
    190                 195                 200
ctc ttg gaa gag aag acr ccg ccg gga atc gtc gat aag ttc ggg tgg     793
Leu Leu Glu Glu Lys Thr Pro Pro Gly Ile Val Asp Lys Phe Gly Trp
205                 210                 215                 220
tgc acg tgg gat gcg ttt tat ttg acg gtg aac cct gac gga gtt cat     841
Cys Thr Trp Asp Ala Phe Tyr Leu Thr Val Asn Pro Asp Gly Val His
                225                 230                 235
aag ggt gtt aag tgt ctc gtc gac ggt ggt tgt ccg ccg gga ttg gtc     889
Lys Gly Val Lys Cys Leu Val Asp Gly Gly Cys Pro Pro Gly Leu Val
            240                 245                 250
cta atc gac gac ggt tgg caa tcg att gga cat gac tcc gat ggt atc     937
Leu Ile Asp Asp Gly Trp Gln Ser Ile Gly His Asp Ser Asp Gly Ile
        255                 260                 265
gat gtt gaa ggg atg agt tgt acc gtc gcc ggg gag caa atg cct tgc     985
Asp Val Glu Gly Met Ser Cys Thr Val Ala Gly Glu Gln Met Pro Cys
    270                 275                 280
agg ctt ctg aaa ttt caa gag aac ttc aag ttc aga gac tac gtc tct    1033
Arg Leu Leu Lys Phe Gln Glu Asn Phe Lys Phe Arg Asp Tyr Val Ser
285                 290                 295                 300
ccg aaa gac aaa aac gaa gtc ggg atg aaa gct ttc gtc aga gat ctg    1081
Pro Lys Asp Lys Asn Glu Val Gly Met Lys Ala Phe Val Arg Asp Leu
                305                 310                 315
aaa gaa gaa ttc tcc acc gtt gat tac atc tac gtc tgg cac gcg ctt    1129
Lys Glu Glu Phe Ser Thr Val Asp Tyr Ile Tyr Val Trp His Ala Leu
            320                 325                 330
tgc ggc tac tgg ggt ggt ctt cgt ccc gga gct cct act ctt ccg ccc    1177
Cys Gly Tyr Trp Gly Gly Leu Arg Pro Gly Ala Pro Thr Leu Pro Pro
        335                 340                 345
tca act att gtc cgg cca gag ctc tcg ccg ggg ctt aag ttg acg atg    1225
Ser Thr Ile Val Arg Pro Glu Leu Ser Pro Gly Leu Lys Leu Thr Met
    350                 355                 360
caa gat ctc gcc gtt gat aag att gtc gat acc gga atc gga ttc gtc    1273
Gln Asp Leu Ala Val Asp Lys Ile Val Asp Thr Gly Ile Gly Phe Val
```

```
      365                 370                 375                 380
      tcg ccg gac atg gcg aat gag ttt tac gaa ggt ctt cac tct cat ctt    1321
      Ser Pro Asp Met Ala Asn Glu Phe Tyr Glu Gly Leu His Ser His Leu
                      385                 390                 395
 5    caa aac gtc ggt att gac ggc gtt aaa gtt gac gtc atc cac ata ttg    1369
      Gln Asn Val Gly Ile Asp Gly Val Lys Val Asp Val Ile His Ile Leu
                  400                 405                 410
      gag atg ttg tgc gag aaa tat ggc ggg aga gta gac ttg gct aaa gct    1417
      Glu Met Leu Cys Glu Lys Tyr Gly Gly Arg Val Asp Leu Ala Lys Ala
10            415                 420                 425
      tac ttc aag gcg tta act tcc tca gtg aat aag cat ttt gac ggt aac    1465
      Tyr Phe Lys Ala Leu Thr Ser Ser Val Asn Lys His Phe Asp Gly Asn
          430                 435                 440
      ggc gtt atc gct agc atg gag cac tgt aat gat ttc atg ttc ctt gga    1513
15    Gly Val Ile Ala Ser Met Glu His Cys Asn Asp Phe Met Phe Leu Gly
      445                 450                 455                 460
      acc gaa gcc atc tct cta ggt cgt gtc ggt gat gac ttt tgg tgc acg    1561
      Thr Glu Ala Ile Ser Leu Gly Arg Val Gly Asp Asp Phe Trp Cys Thr
                      465                 470                 475
20    gat cca tca ggc gac ata aac ggc aca tat tgg ctg caa gga tgc cac    1609
      Asp Pro Ser Gly Asp Ile Asn Gly Thr Tyr Trp Leu Gln Gly Cys His
                  480                 485                 490
      atg gtc cac tgt gcc tac aac agt ctt tgg atg gga aat ttc atc cag    1657
      Met Val His Cys Ala Tyr Asn Ser Leu Trp Met Gly Asn Phe Ile Gln
25            495                 500                 505
      cct gat tgg gac atg ttt cag tcc aca cat cct tgt gct gag ttc cat    1705
      Pro Asp Trp Asp Met Phe Gln Ser Thr His Pro Cys Ala Glu Phe His
          510                 515                 520
      gct gct tct cgt gcc atc tcc ggt ggg ccc att tac atc agc gat tgt    1753
30    Ala Ala Ser Arg Ala Ile Ser Gly Gly Pro Ile Tyr Ile Ser Asp Cys
      525                 530                 535                 540
      gtg ggc cag cac gat ttc gat ctc ttg aag cga ctc gtc ttg cct gac    1801
      Val Gly Gln His Asp Phe Asp Leu Leu Lys Arg Leu Val Leu Pro Asp
                      545                 550                 555
35    ggt tcg att ttg agg tgt gag cac tat gca ctc cca act cgt gac cgt    1849
      Gly Ser Ile Leu Arg Cys Glu His Tyr Ala Leu Pro Thr Arg Asp Arg
                  560                 565                 570
      ctc ttt gaa gac cct ctt cat gat ggc aaa acc atg ctc aag att tgg    1897
      Leu Phe Glu Asp Pro Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp
```

```
          575                 580                 585
aac ttg aac aag tac act gga att att gga gca ttc aac tgc caa gga   1945
Asn Leu Asn Lys Tyr Thr Gly Ile Ile Gly Ala Phe Asn Cys Gln Gly
    590                 595                 600
gga gga tgg tgc aga gaa acc cga cgc aac caa tgc ttc tcc caa tgc   1993
Gly Gly Trp Cys Arg Glu Thr Arg Arg Asn Gln Cys Phe Ser Gln Cys
605                 610                 615                 620
gtt aac acg tta acc gcc aca aca aat cct aag gac gtt gaa tgg aac   2041
Val Asn Thr Leu Thr Ala Thr Thr Asn Pro Lys Asp Val Glu Trp Asn
                625                 630                 635
agt ggg aac aac cca atc tcc gtt gaa aac gtt gaa gag ttt gct ttg   2089
Ser Gly Asn Asn Pro Ile Ser Val Glu Asn Val Glu Glu Phe Ala Leu
            640                 645                 650
ttc ttg tct cag tct aag aag ctt gtg ttg tct gga cca aac gat gat   2137
Phe Leu Ser Gln Ser Lys Lys Leu Val Leu Ser Gly Pro Asn Asp Asp
        655                 660                 665
ctc gag atc act ttg gag cct ttc aag ttt gag cta atc act gtc tca   2185
Leu Glu Ile Thr Leu Glu Pro Phe Lys Phe Glu Leu Ile Thr Val Ser
    670                 675                 680
cca gtt gtc act att gag ggt agt tcg gtt cag ttt gct cca atc gga   2233
Pro Val Val Thr Ile Glu Gly Ser Ser Val Gln Phe Ala Pro Ile Gly
685                 690                 695                 700
ttg gtt aac atg cta aac act agc ggt gca att cga tcc ttg gtg tat   2281
Leu Val Asn Met Leu Asn Thr Ser Gly Ala Ile Arg Ser Leu Val Tyr
                705                 710                 715
cat gag gaa tcc gtt gag att gga gtt cgt ggt gct gga gag ttc agg   2329
His Glu Glu Ser Val Glu Ile Gly Val Arg Gly Ala Gly Glu Phe Arg
            720                 725                 730
gtt tat gca tca agg aaa cct gcg agc tgc aaa att gat ggt gaa gtt   2377
Val Tyr Ala Ser Arg Lys Pro Ala Ser Cys Lys Ile Asp Gly Glu Val
        735                 740                 745
gtt gag ttt gga tac gaa gag tca atg gtg atg gtt caa gtg cct tgg   2425
Val Glu Phe Gly Tyr Glu Glu Ser Met Val Met Val Gln Val Pro Trp
    750                 755                 760
tct gca ccc gag ggt ttg tct tct att aag tat gag ttt tag agtttccga 2476
Ser Ala Pro Glu Gly Leu Ser Ser Ile Lys Tyr Glu Phe
765                 770                 775
aggtgcttat ttgtatcctt ctaaactcct taattatgag ctccgtgccg tttctttttc 2536
tatatggttt ctgagagtga acatctaata tttacccact agggtataat tattggcttt 2596
```

taagtgattt gtttttgaac tgtttttagt ggtgtaattt gtactgcccc tattattttt 2656
catatttatt tgtgaaagat aaaaaaaaaa aaaa 2690

<210> 7
<211> 572
<212> PRT
<213> Brassica napus

<400> 7

Leu Glu Glu Lys Thr Pro Pro Gly Ile Val Asp Lys Phe Gly Trp Cys
5 10 15
Thr Trp Asp Ala Phe Tyr Leu Thr Val Asn Pro Asp Gly Val His Lys
20 25 30
Gly Val Lys Cys Leu Val Asp Gly Gly Cys Pro Pro Gly Leu Val Leu
35 40 45
Ile Asp Asp Gly Trp Gln Ser Ile Gly His Asp Ser Asp Gly Ile Asp
50 55 60
Val Glu Gly Met Ser Cys Thr Val Ala Gly Glu Gln Met Pro Cys Arg
65 70 75 80
Leu Pro Lys Phe Gln Glu Asn Phe Lys Phe Arg Asp Tyr Val Ser Pro
85 90 95
Lys Asp Lys Asn Glu Val Gly Met Lys Ala Phe Val Arg Asp Leu Lys
100 105 110
Glu Glu Phe Ser Thr Val Asp Tyr Ile Tyr Val Trp His Ala Leu Cys
115 120 125
Gly Tyr Trp Gly Gly Leu Arg Pro Gly Ala Pro Thr Leu Pro Pro Ser
130 135 140
Thr Ile Val Arg Pro Glu Leu Ser Pro Gly Leu Lys Leu Thr Met Gln
145 150 155 160
Asp Leu Ala Val Asp Lys Ile Ile Asp Thr Gly Ile Gly Phe Val Ser
165 170 175
Pro Asp Met Ala Asn Glu Phe Tyr Glu Gly Leu His Ser His Leu Gln
180 185 190
Asn Val Gly Ile Asn Gly Val Lys Val Asp Val Ile His Ile Leu Glu
195 200 205
Met Leu Cys Glu Lys Tyr Gly Gly Arg Val Asp Leu Ala Lys Ala Tyr
210 215 220
Phe Lys Ala Leu Thr Ser Ser Val Asn Lys His Phe Asp Gly Asn Ala
225 230 235 240

Val Ile Ala Ser Met Glu His Cys Asn Asp Phe Met Phe Leu Gly Thr
245 250 255
Glu Ala Ile Ser Leu Gly Arg Val Gly Asp Asp Phe Trp Cys Thr Asp
260 265 270
Pro Ser Gly Asp Ile Asn Gly Thr Tyr Trp Leu Gln Gly Cys His Met
275 280 285
Val His Cys Ala Tyr Asn Ser Leu Trp Met Gly Asn Phe Ile Gln Pro
290 295 300
Asp Trp Asp Met Phe Gln Ser Thr His Pro Cys Ala Glu Phe His Ala
305 310 315 320
Ala Ser Arg Ala Ile Ser Gly Gly Pro Ile Tyr Ile Ser Asp Cys Val
325 330 335
Gly Gln His Asp Phe Asp Leu Leu Arg Arg Leu Val Leu Pro Asp Gly
340 345 350
Ser Ile Leu Arg Cys Glu Tyr Tyr Ala Leu Pro Thr Arg Asp Arg Leu
355 360 365
Phe Glu Asp Pro Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp Asn
370 375 380
Leu Asn Lys Tyr Thr Gly Ile Ile Gly Ala Phe Asn Cys Gln Gly Gly
385 390 395 400
Gly Trp Cys Arg Glu Thr Arg Arg Asp Gln Cys Phe Ser Gln Cys Val
405 410 415
Asn Thr Leu Thr Ala Thr Thr Asn Pro Asn Asp Val Glu Trp Asn Ser
420 425 430
Gly Asn Asn Pro Ile Ser Ile Glu Asn Val Glu Glu Phe Ala Leu Phe
435 440 445
Leu Ser Gln Ser Lys Lys Leu Val Leu Ser Gly Gln Asn Asp Asp Leu
450 455 460
Glu Ile Thr Leu Glu Pro Phe Lys Phe Glu Leu Ile Thr Val Ser Pro
465 470 475 480
Val Val Thr Ile Glu Gly Ser Ser Val Gln Phe Ala Pro Ile Gly Leu
485 490 495
Val Asn Met Leu Asn Thr Ser Gly Ala Ile Arg Ser Leu Val Tyr His
500 505 510
Glu Glu Ser Val Glu Ile Gly Val Arg Gly Ala Gly Glu Phe Arg Val
515 520 525
Tyr Ala Ser Lys Lys Pro Val Ser Cys Lys Ile Asp Gly Glu Asp Val
530 535 540
Glu Phe Gly Tyr Glu Glu Ser Met Val Met Val Gln Val Pro Trp Ser

545 550 555 560
Ala Pro Glu Gly Leu Ser Ser Ile Lys Tyr Leu Phe
565 570 572

<210> 8
<211> 1762
<212> DNA
<213> Brassica napus

<220>
<221> CDS
<222> (1)...(1719)

<400> 8

```
ttg gaa gaa aaa acg ccg ccg gga atc gtc gat aag ttc ggg tgg tgc      48
Leu Glu Glu Lys Thr Pro Pro Gly Ile Val Asp Lys Phe Gly Trp Cys
                  5                  10                  15
acg tgg gat gcg ttt tat ttg acg gtg aac cct gac gga gtt cat aag      96
Thr Trp Asp Ala Phe Tyr Leu Thr Val Asn Pro Asp Gly Val His Lys
             20                  25                  30
ggt gtt aag tgt ctc gtc gac ggt ggt tgt ccg ccg gga ttg gtc cta     144
Gly Val Lys Cys Leu Val Asp Gly Gly Cys Pro Pro Gly Leu Val Leu
         35                  40                  45
atc gac gac ggt tgg caa tcg att gga cat gac tcc gat ggt atc gat     192
Ile Asp Asp Gly Trp Gln Ser Ile Gly His Asp Ser Asp Gly Ile Asp
     50                  55                  60
gtt gaa ggg atg agt tgt acc gtc gcc ggg gag caa atg cct tgc agg     240
Val Glu Gly Met Ser Cys Thr Val Ala Gly Glu Gln Met Pro Cys Arg
 65                  70                  75                  80
ctt ccg aaa ttt caa gag aac ttc aag ttc aga gac tac gtc tct ccg     288
Leu Pro Lys Phe Gln Glu Asn Phe Lys Phe Arg Asp Tyr Val Ser Pro
                 85                  90                  95
aaa gac aaa aac gaa gtc ggg atg aaa gct ttc gtc aga gat ctg aaa     336
Lys Asp Lys Asn Glu Val Gly Met Lys Ala Phe Val Arg Asp Leu Lys
            100                 105                 110
gaa gaa ttc tcc acc gtt gat tac atc tac gtc tgg cac gcg ctt tgc     384
Glu Glu Phe Ser Thr Val Asp Tyr Ile Tyr Val Trp His Ala Leu Cys
        115                 120                 125
ggy tac tgg ggw ggt ctt cgt ccc gga gct cct act ctt ccg ccs tcr     432
```

```
Gly Tyr Trp Gly Gly Leu Arg Pro Gly Ala Pro Thr Leu Pro Pro Ser
    130                 135                 140
act att gtc cgr cca gag ctc tcg ccg ggg ctt aag ttg acg atg caa   480
Thr Ile Val Arg Pro Glu Leu Ser Pro Gly Leu Lys Leu Thr Met Gln
145                 150                 155                 160
gat ctc gcc gtt gat aag atc atc gat acc gga atc gga ttc gtc tcg   528
Asp Leu Ala Val Asp Lys Ile Ile Asp Thr Gly Ile Gly Phe Val Ser
                165                 170                 175
ccg gac atg gcg aac gag ttt tac gaa ggt ctt cac tct cat ctt caa   576
Pro Asp Met Ala Asn Glu Phe Tyr Glu Gly Leu His Ser His Leu Gln
            180                 185                 190
aac gtc ggc att aac ggc gtt aaa gtt gac gtt atc cac ata ctg gag   624
Asn Val Gly Ile Asn Gly Val Lys Val Asp Val Ile His Ile Leu Glu
        195                 200                 205
atg ttg tgc gag aaa tat ggc ggg aga gtt gac ttg gct aaa gct tac   672
Met Leu Cys Glu Lys Tyr Gly Gly Arg Val Asp Leu Ala Lys Ala Tyr
    210                 215                 220
ttc aag gcg tta acg tcg tca gtg aat aag cat ttt gac ggc aac gcc   720
Phe Lys Ala Leu Thr Ser Ser Val Asn Lys His Phe Asp Gly Asn Ala
225                 230                 235                 240
gtt atc gcc agc atg gag cac tgt aat gac ttc atg ttc ctt gga acc   768
Val Ile Ala Ser Met Glu His Cys Asn Asp Phe Met Phe Leu Gly Thr
                245                 250                 255
gaa gcc atc tct cta ggt cgt gtc ggt gat gac ttt tgg tgc acg gat   816
Glu Ala Ile Ser Leu Gly Arg Val Gly Asp Asp Phe Trp Cys Thr Asp
            260                 265                 270
cca tct ggc gac att aac ggc acg tat tgg ctg caa gga tgt cac atg   864
Pro Ser Gly Asp Ile Asn Gly Thr Tyr Trp Leu Gln Gly Cys His Met
        275                 280                 285
gtc cac tgt gcc tac aac agt ctt tgg atg gga aat ttc atc cag cct   912
Val His Cys Ala Tyr Asn Ser Leu Trp Met Gly Asn Phe Ile Gln Pro
    290                 295                 300
gat tgg gac atg ttt cag tcc aca cat cct tgt gct gag ttc cat gct   960
Asp Trp Asp Met Phe Gln Ser Thr His Pro Cys Ala Glu Phe His Ala
305                 310                 315                 320
gct tca cgt gcc atc tcc ggt ggg ccc att tac atc agc gat tgt gtg  1008
Ala Ser Arg Ala Ile Ser Gly Gly Pro Ile Tyr Ile Ser Asp Cys Val
                325                 330                 335
ggc cag cac gat ttc gat ctc ttg agg aga ctc gtt ttg cct gac ggt  1056
```

```
     Gly Gln His Asp Phe Asp Leu Leu Arg Arg Leu Val Leu Pro Asp Gly
                 340                 345                 350
     tcg att ttg agg tgt gag tac tat gct ctc cca act cgt gac cgt ctc    1104
     Ser Ile Leu Arg Cys Glu Tyr Tyr Ala Leu Pro Thr Arg Asp Arg Leu
          355                 360                 365
     ttt gaa gac cct ctt cat gat ggc aaa acc atg ctc aag att tgg aac    1152
     Phe Glu Asp Pro Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp Asn
     370                 375                 380
     ttg aac aag tac act gga atc atc gga gca ttc aac tgt caa gga gga    1200
     Leu Asn Lys Tyr Thr Gly Ile Ile Gly Ala Phe Asn Cys Gln Gly Gly
     385                 390                 395                 400
     gga tgg tgc aga gaa act cga cgc gac caa tgc ttc tcc caa tgc gtt    1248
     Gly Trp Cys Arg Glu Thr Arg Arg Asp Gln Cys Phe Ser Gln Cys Val
                         405                 410                 415
     aac acg tta acc gcc aca aca aat cct aat gac gtt gaa tgg aac agt    1296
     Asn Thr Leu Thr Ala Thr Thr Asn Pro Asn Asp Val Glu Trp Asn Ser
                 420                 425                 430
     ggg aac aac ccg atc tcc att gaa aac gtt gaa gag ttt gct ttg ttc    1344
     Gly Asn Asn Pro Ile Ser Ile Glu Asn Val Glu Glu Phe Ala Leu Phe
          435                 440                 445
     ttg tct caa tcc aag aag ctt gtg ttg tcc ggg caa aac gat gat ctc    1392
     Leu Ser Gln Ser Lys Lys Leu Val Leu Ser Gly Gln Asn Asp Asp Leu
     450                 455                 460
     gag atc aca tta gag ccc ttc aag ttc gag ctc atc act gtc tca cca    1440
     Glu Ile Thr Leu Glu Pro Phe Lys Phe Glu Leu Ile Thr Val Ser Pro
     465                 470                 475                 480
     gtt gtc acc att gag ggc agt tcg gtt cag ttt gct cca atc gga ttg    1488
     Val Val Thr Ile Glu Gly Ser Ser Val Gln Phe Ala Pro Ile Gly Leu
                         485                 490                 495
     gtt aac atg ctt aac act agc ggt gcg att cga tcc ttg gtt tat cat    1536
     Val Asn Met Leu Asn Thr Ser Gly Ala Ile Arg Ser Leu Val Tyr His
                 500                 505                 510
     gag gaa tcc gtt gag atc ggt gtt cgt ggt gct gga gaa ttc agg gtt    1584
     Glu Glu Ser Val Glu Ile Gly Val Arg Gly Ala Gly Glu Phe Arg Val
          515                 520                 525
     tat gca tcg aag aaa cct gtg agc tgc aag att gat ggt gaa gat gtt    1632
     Tyr Ala Ser Lys Lys Pro Val Ser Cys Lys Ile Asp Gly Glu Asp Val
     530                 535                 540
     gag ttt ggg tac gaa gag tca atg gtg atg gtt caa gtg cct tgg tct    1680
```

Glu Phe Gly Tyr Glu Glu Ser Met Val Met Val Gln Val Pro Trp Ser
545 550 555 560
gca cca gag ggt ttg tct tct att aag tat ttg ttt tag agttatttaa 1729
Ala Pro Glu Gly Leu Ser Ser Ile Lys Tyr Leu Phe
565 570
ggtgcttaat tgaaaaaaaa aaaaaaaaaa aaa 1762

<210> 9
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 9
ccaatctgat catgcttgtg ccgaa 25

<210> 10
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 10
ggaacaaagt tatgcactat tatttaaggt 30

<210> 11
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 11
ctaccaaatt ccacaactta aagttca 27

<210> 12
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 12
ggaataataa gcttcacaca tactgtactc tc 32

<210> 13
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obatin raffinose synthase gene.

<400> 13
atggctccaa gctttagcaa ggaaaattcc 30

<210> 14
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 14
tcaaaataag tactcaacag tggtaaaacc 30

<210> 15
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 15
ttggaagaga agacgccgcc gggaatcgtc 30

<210> 16
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 16
ttaagccccg gcgagagctc tggccggaca 30

<210> 17
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 17
accaatccaa aatctcatca aataatcgca 30

<210> 18
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obatin raffinose synthase gene.

<400> 18
aaataatagg ggcagtacaa attacaccac 30

<210> 19
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 19
atggctccac cgagcgtaat taaatccga 29

<210> 20
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 20
ctaaaactca tacttaatag aagacaaacc 30

<210> 21
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i.

<400> 21
cgatggatgg gnaanttnat ncanccngan tggganatgt t 41

<210> 22
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

<400> 22
ggccacatnt tnacnarncc natnggngcn aa 32

<210> 23
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 23
tgttactagg cgaaacaaga gtagctctga 30

<210> 24
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i.

<400> 24
cgaggnggnt gnccnccngg nttngtnatn atngangang gntggca 47

<210> 25
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, y is t or c, r is a or g.

<400> 25
atyttrtcna cngcnarrtc ytccatngt 29

<210> 26
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, y is t or c.

<400> 26
ggnacntant ggytncangg ntgncanatg gtncantg 38

<210> 27
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

April, 16, E.W.

April. 16, K.O.

<400> 27
ggccacatnt tnacnarncc natnggngcn aa 32

<210> 28
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 28
atctatttgt catgacgatg atccga 26

<210> 29

<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 29
ggccctcatt cccatattgg gatgatcctc 30

<210> 30
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 30
aagcatgcca aacatacaca tgctcaacag 30

<210> 31
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 31
agacccgggg aaagctttgg ggttactact 30

<210> 32
<211> 28
<212> DNA
<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 32
tggatgggaa actttataca ccctgact 28

<210> 33
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 33
gacatgttcc catctacaca cccttgtg 28

<210> 34
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 34
ccaatttatg ttagtgatgt tgttggcaag 30

<210> 35
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 35
tcgactccca gggtagaatt gtcatc 26

<210> 36

<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i.

<400> 36
cgattnaang tntggtggac nacncantgg gtngg 35

<210> 37
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

<400> 37
cantgnacca tntgncancc ntgnarccan tangtncc 38

<210> 38
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 38
gttagggttc atatgaacac cttcaagctc 30

<210> 39
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 39
caacggcgag atcttgcatc gtcaac 26

<210> 40
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 40
ggattcgaca caaaccgcca cgtcatcgtc 30

<210> 41
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 41
ccacgtgcac cacccgaact tatcgac 27

<210> 42
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 42
aacatcgata ccatcggagt catgtccaat 30

<210> 43
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 43
gttagggttc atatgaacac cttcaagctc 30

<210> 44
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 44
tctacgtctg gcacgcgctt tgcggctac 29

<210> 45
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 45
gttgacgtca tccacatatt ggagatgttg t 31

<210> 46
<211> 29
<212> DNA
<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 46
gttatcgcta gcatggagca ctgtaatga 29

<210> 47
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 47
aacgagctca atccaaaatc tcatcaaata atcgc 35

<210> 48
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 248
acaatagttg agggcggaag agtag 25

<210> 49
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide linker to obtain raffinose synthase gene.

<400> 49
gatcgagctc gtgtcggatc cagct 25

<210> 50

<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide linker to obtain raffinose synthase gene.

<400> 50
ggatccgaca cgagctc 17

<210> 51
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to confirm direction of the inserted raffinose synthase gene.

<400> 51
cctcctcgga ttccattgcc cagctatctg 30

<210> 52
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to confirm direction of the inserted raffinose synthase gene.

<400> 52
ggattcgaca caaaccgcca cgtcatcgtc 30

<210> 53
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide primer to confirm direction of the inserted raffinose synthase gene.

<400> 53
tctacgtctg gcacgcgctt tgcggctac 29